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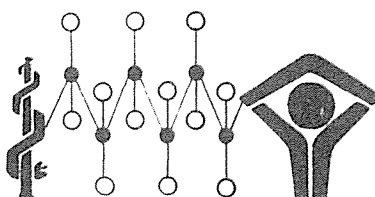
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International Symposium
Perth — Western Australia
5th-9th February 1989

WORKSHOP PROCEEDINGS

ADVANCES IN BIOMEDICAL POLYMERS

Australian Foundation for Prevention of
Blindness — Lions Eye Institute

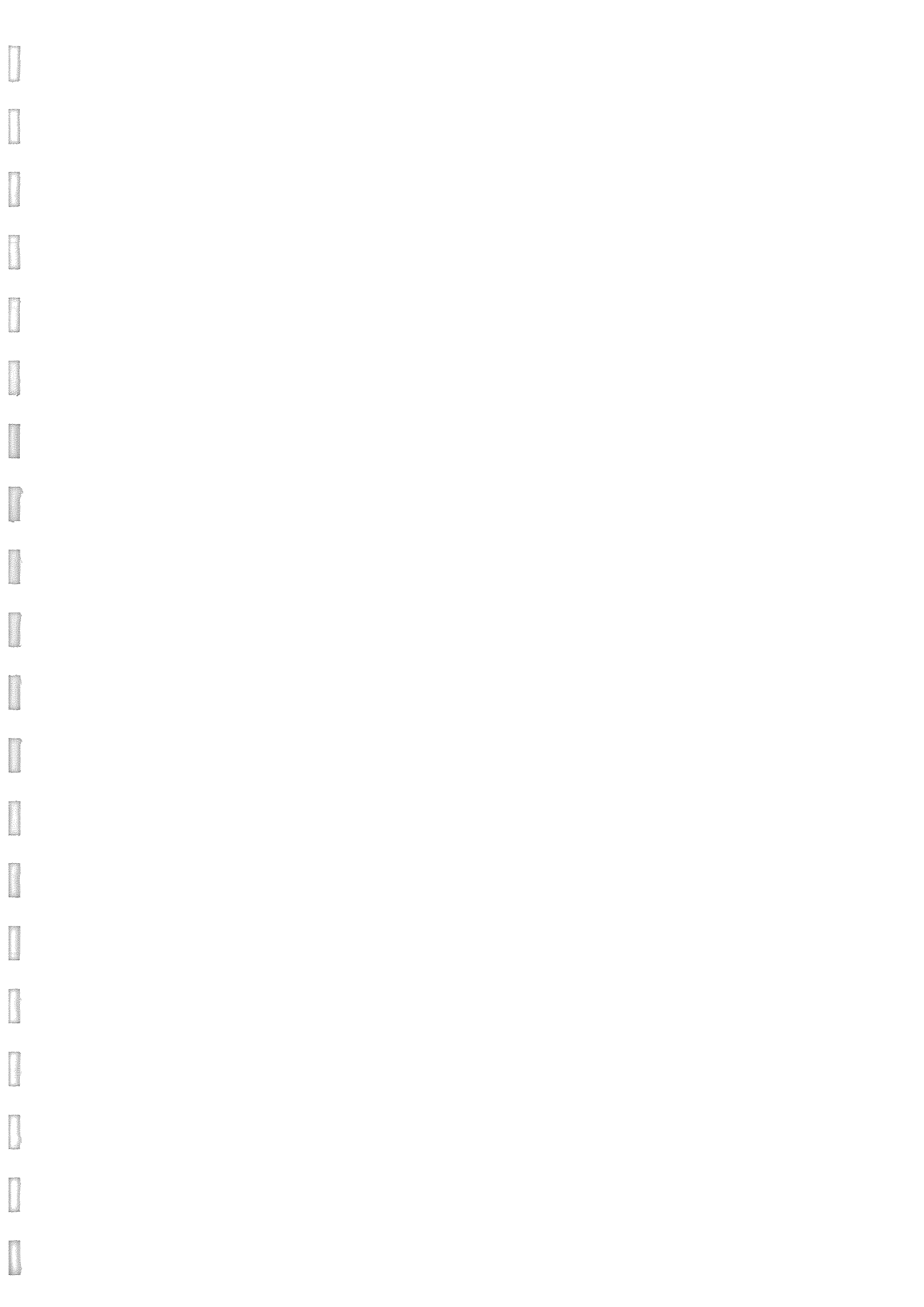


and

Royal Australian Chemical Institute
Western Australian Polymer Group



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ADVANCES IN BIOMEDICAL POLYMERS SYMPOSIUM

WORKSHOP

BIODEGRADATION AND BIOCOMPATIBILITY
of Biomaterials

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INTRODUCTION:

A conference on biomaterials cannot adequately describe new technology without covering the very important topics of biodegradability and biocompatibility. This workshop is planned to draw attention to these very important criteria in synthetic material design.

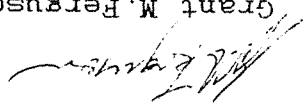
Material performance is dependent on the environment in which it must function. Human nature, more often than not, demands much more of a material than its design criteria allow. The human body is an extremely harsh environment for a synthetic material to perform to excellence. However, armed with a knowledge of the service environment, a polymer can be tailor made to give maximum performance.

The environmental impact on synthetic materials, both in the macrosphere and the biological microsphere, is a young science. I can recall examining a sheet Hypalon reservoir cover a few years ago which suffered extensive failure within a few months of service. A combination of physical stress and ultra-violet radiation triggered the degradation process which culminated in large scale biodegradation. The biodegradation was caused by large microorganism populations existing in pooled surface water attacking the physically degraded polymer. The polymer surface in contact with chemically treated reservoir water was in excellent condition. The biodegradation of this particular Hypalon exacerbated the specific environmental impact.

Problems encountered in material performance are most often related to the vast number of environmental variables. These variables are difficult to assess in isolation and in combination. Full assessment can only be achieved on an individual basis since each physiological environment can be regarded as unique.

For a material to be clinically

biocompatible it should be non-toxic, non-immunogenic, non-degradable, if degradable then the material must be easily removed from the body and should not physically fail in service. The material must exist in biological harmony with its physiological environment. New technologies developed to overcome some of the limitations of synthetic materials will be discussed in this workshop. The concluding discussion session will highlight areas of concern for future research. Synthetic materials have an important role to play in improving the quality and quantity of life for mankind well into the future.



Grant M. Ferguson

Chairman

Western Australian Polymer Group
Royal Australian Chemical Institute

GENERAL ANNOUNCEMENTS

The organizing committee reserves the right to alter the programme. All programme changes will be displayed on the noticeboards adjacent to the registration desk. PLEASE CHECK THE NOTICEBOARDS.

Authors should check the starting time/s for their paper/s. The programme is made up from 20 minute slots. Contributed papers have been allocated one slot, invited papers two slots and plenary papers three slots. The time slots include introduction and question time. For example a 20 minute slot should allow authors 15 minutes paper presentation time.

Authors of paper presentations are requested to attend the lecture theatre 15 minutes before the commencement of the session in which their paper is to be presented. This is to make sure the projectionist has

slides etc. set up in accordance with the speakers wishes and for the session Chairman to issue any last minute instructions.

Papers will start and finish promptly at the advertised times. Posters will be displayed for the duration of the conference. Poster authors are requested to attend their posters during the advertised poster session times.

To gain entry to Workshop and Symposium lectures and other functions ALL delegates are requested to wear the name badges issued at registration.



WORKSHOP PROGRAMME
ADVANCES IN BIOMEDICAL POLYMERS SYMPOSIUM

REGISTRATION

5.00pm - 6.00pm: Saturday 4 February, 1989.
8.30am - 8.50am: Sunday 5 February, 1989.

Chairman - Grant M. Ferguson.

SESSION 1. BIODEGRADABILITY.

9.00am - 9.45am:

Roger B. Marchant (Dept. Biomedical Engineering, Case Western Reserve University, Ohio, USA) - "On The Biodegradation of Polymeric Materials Intended For Long-term Biomedical Applications".

9.45 - 10.15am: Morning tea

Chairman - Bill Reid

SESSION 2. BIOCOMPATIBILITY.

10.15 - 11.00am:

Stuart L. Cooper (Dept. Chem. Eng. U. Wisconsin, USA) - "Polyurethanes at the Biological Interface".

11.00 - 11.45am:

George B. Butler (Chemistry Dept., University of Florida, Gainesville, USA) - "Anti-tumor Activity of Polyanions From Copolymers Related to Diveona".

11.45 - 1.00pm: Lunch

Chairman - Geoff Richardson

SESSION 3. BIODEGRADABILITY.

1.00 - 1.45pm:

Jorge Heller (SRI International, Menlo Park, California, USA) - "Controlled Drug Release from Biodegradable Implants".

1.45 - 2.30pm

Bruce K. Milthorpe and K. Schindhelm (Biomedical Engineering U. NSW, Aust.) - "Polymers for Vascular Grafts"

2.30 - 3.00pm: Afternoon tea.

Chairman - Ed Scull

SESSION 4. BIOCOMPATIBILITY.

3.00 - 3.45pm:

David S. Breslow (Chemistry Dept., University of Florida, Gainesville, USA) - "MVE a Biologically Active Synthetic Anticancer Polymer".

3.45pm - 5.00pm: Panel Discussion.

5.00pm - 6.00pm: Symposium Registration

6.30pm - 8.30pm WELCOMING COCKTAIL

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ON THE BIODEGRADATION OF POLYMERIC
MATERIALS INTENDED FOR LONG-TERM
BIOMEDICAL APPLICATIONS

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SUMMARY

Biocompatibility and biodegradation of implanted biomedical polymers are closely related phenomena, largely determined by the interfacial host-biomaterial reactions and interactions. This presentation will review the potential for biomedical polymers, intended for long-term cardiovascular and/or soft-tissue applications, to undergo biodegradation in the biological environment. The macroscopic effects of biodegradation on polymer properties and performance are well-documented, but the underlying mechanisms are often not known because of our incomplete understanding of how a complex (biological) system interacts with a complex (polymer) system. Consequently, progress in this area depends on identifying and studying the important components in the biological environment and in the biomedical polymers that may contribute to the observed biodegradation. Factors that may account for the accelerated degradation observed with many implanted polymers, including potential host-mediated degradation mechanisms and the effects of biodegradation on biocompatibility, will be reviewed and discussed.

INTRODUCTION

The use of synthetic polymers in cardiovascular and extravascular prostheses has increased considerably in recent years, (see Table I) and is continuing to grow at a rapid rate. These polymer materials are often required to perform for extended periods of time in the host environment. Consequently, the ability to resist biodegradation, and thus maintain optimum functional performance long after implantation, is an important property for any polymer intended for long-term biomedical applications.

Unfortunately, evidence for biodegradation of many biomedical polymers in vitro, in vivo, and regrettably under clinical conditions is now well-established. Segmented polyurethanes, polyesters, polyamides, polyolefins and polysiloxane elastomers all have been shown to deteriorate in vivo, often at much faster rates than observed under in vitro conditions. However, in most cases the rates of performance deterioration are slow enough to encourage the continued application of the polymers, but fast enough to stimulate concern and a considerable research effort on the part of numerous independent research groups. These concerns are more than justified by the potential consequences of a failed biomedical prosthetic device. The potential hazards from undesirable biodegradation may have particularly serious sequelae. Localized areas of surface degradation may lead to initiation sites for thrombus formation, or for calcification; or the release locally and systemically, of possibly toxic degradation products. Mechanically, degradation will lead to the propagation of surface-initiated cracks and ultimately to life-threatening failure.

TABLE I

SOME
MEDICAL APPLICATIONS OF POLYMERS

APPLICATIONS	POLYMERS
CARDIOVASCULAR SYSTEM	
CATHETERS, CHRONIC SHUNTS,	POLYOLEFINS, PDMS,
HEART VALVES, TOTAL HEART	PTFE, POLYURETHANES,
COMPONENTS, VENTRICULAR	POLYCARBONATE, GELATIN,
ASSIST, VASCULAR GRAFTS,	POLYESTERS, PAN &
HEMODIALYSIS, OXYGENATORS	CELLULOSE MEMBRANES
SOFT TISSUE PROSTHESES	
RECONSTRUCTIVE PROSTHESES;	PTFE, PDMS, SILICONES
MAMMARY PROSTHESES;	POLYURETHANES, NYLONS
ARTIFICIAL CARTILAGE, URETER	HYDROGELS, POLYPEPTIDES
BLADDER, SKIN; TISSUE	COLLAGEN, POLYETHYLENE,
PATCHES, SUTURES, INTERNAL	POLYPROPYLENE, VARIOUS
SHUNTS & TUBING; DRUG DELIVERY	COPOLYMERS, ACRYLICS
SYSTEMS, CONTACT LENSES,	
CORNEAL PROSTHESES, INTRAOCULAR	
LENS, NEURAL STIMULATION	
SYSTEMS.	

In the context of clinical evaluation of biomedical polymers, biodegradation phenomena may be described as the premature deterioration and/or failure of an implanted biomedical device. This attributes the cause of failure to some unknown biological agent or bio-mediated process. From this limited perspective,

the nature of the biodegradation, may be a physical, mechanical or chemical/biochemical derived process. Much of the useful information on polymer performance has been derived from studies focused on the clinical evaluation of retrieved implants and on qualitative in vivo comparisons between different commercially available materials. Progress on a more fundamental level has been hindered by the absence of established mechanisms and development of appropriate quantitative in vitro model systems. This situation is largely a consequence of our current lack of understanding of host-material interfacial reactions and interactions.

HOST EFFECTS ON POLYMER PERFORMANCE

There are many host factors that may affect the performance of biomedical polymers. In addition to bio-induced chemical effects, material performance can be affected by absorption/adsorption of water, ions, lipids and proteins; desorption/extraction of polymer additives such as stabilizers; mechanical deformation, such as long-term cyclic loading; Also, the effects of fabrication and sterilization procedures can affect material performance. In general, physical and mechanical effects are much easier to quantify than chemical effects, unless there is synergism between processes. In many cases, it is still not clear as to which of the constituent factors are the most important, and what are the crucial interactions between the biological environment and the respective synthetic polymer that accelerate the observed decrease in macroscopic properties.

PTFE and PDMS
Two chemically stable polymers that are used for biomedical applications are polydimethylsiloxane (PDMS) elastomers and polytetrafluoroethylene (PTFE). The biostability of PTFE is well-established, although the restricted range of mechanical properties tends to limit its potential applications, except when in the expanded Gortex form. Crosslinked PDMS elastomers are usually compounded with silica to reinforce its relatively poor mechanical properties (1). Silica is cytotoxic, but there does not appear to be any significant evidence for its release from the polymer (2). PDMS elastomers have been used for many biomedical applications and offer an alternative to the polyurethane elastomers. Several

studies have shown that these PDMS polymers are stable for long periods in vivo (1,2). However, when subjected to mechanical deformation in contact with blood the deterioration in properties can be significant (3,4). This mechanically induced effect is generally associated with polymer swelling caused by an initial absorption of plasma lipids (4), eventually resulting in material failure. In the absence of mechanical deformation, lipid absorption is usually less than 2% with mechanical properties showing little change even after long periods of implantation (1).

Polyolefins

In the absence of high temperatures and ultraviolet light, one would expect polyolefins to demonstrate high resistance to a biological environment. This prediction is generally observed. However, there have been examples of implanted polyolefins showing degradation behavior (5). The mechanism has been suggested (6) to be similar in nature to the peroxidation chain reactions that occur in air (7), which leads to discoloration, embrittlement and mechanical failure. A potential problem with the use of polyolefins is that a substantial decrease in mechanical performance can occur after very low levels of surface oxidation, because the degradation is usually concentrated in the amorphous phase (8).

Polyurethanes

Segmented Polyurethanes are a diverse family of polymers and their significance as biomaterials is derived from a wide range of useful properties that can be incorporated into the polymers by adjusting the macromolecular composition and morphology which results in a wide variation in chemical, physical, surface and mechanical properties (9). As a result, polyurethanes have found use in a wide range of biomedical applications. The clinical manifestation of biodegradation has been most clearly identified in the macroscopic deterioration of polyetherurethane pacemaker leads, where surface cracking of the polymer with high current drain through the PEU insulation has been observed (10). These clinical observations have lead to a large number of in vitro and in vivo studies on the biodegradation of various polyurethanes (10-14). The biodegradation appears to be

chemical in nature, but the mechanism, which the degradation occurs has not been unequivocally elucidated. While the effects of implantation on the properties of polyurethanes have been reported extensively, chemical analysis of the biodegradation has proven very difficult, because the rates of biodegradation are relatively slow, sorption processes often interfere with any chemical analysis of retrieved polymer implants, the composition of the surface of the polyurethanes will undergo some rearrangement when exposed to an aqueous environment, and in vitro studies suffer from not knowing the precise nature of the biological reactants and catalysts that cause the biodegradation.

Other Biomedical Polymers

Polyesters (15), polyamides (16) and other synthetic polymers with hydrolyzable groups have shown evidence for significant biodegradation behavior after implantation. For example, the mechanical failure of Dacron arterial prostheses is now well-documented. In a recent study, Vinard et al. (15) showed that the stress at break of 22 retrieved poly(ethylene terephthalate) (PET) commercial vascular grafts were all diminished (2-75% decreases were observed). The decreases in mechanical strength were accompanied by concomitant decreases in polymer crystallinity, as determined by X-ray diffraction and DSC measurements. These data appeared to be generally consistent with those reported previously. While the detrimental effects of implantation on the properties of PET have been demonstrated, the underlying mechanisms need to be studied and elucidated.

HOST-POLYMER INTERACTIONS

In blood contacting devices surface induced mechanisms are of critical importance. These can include initiation of the clotting and complement cascades as well as activation of circulating platelets and leukocytes. However, a polymer surface can be almost as complex as the biological environment with which it is interacting. There are many aspects of a polymer which may perturb the two-way dynamic process between implant and host (Table II). A polymer surface will include a basic equilibrium composition which may be different from the bulk composition (e.g., segmented polyurethanes). The polymer may be

A relatively small but significant simplification from the blood-polymer interactions is to focus on the interactions in a soft-tissue extravascular site. While still highly complex, this conveniently minimizes some of the constituent variables such as platelet and erythrocyte interactions and coagulation factors. At interfaces with soft-tissue, the host will react to the presence of the polymer through the well-characterized mechanism of inflammation and wound healing (Figure 1). The initial response involves hemodynamic changes and increased vascular permeability close to an implant site. This promotes the transport of protein-rich fluid, including molecular mediators (e.g., complement factors) and phagocytic leukocytes (macrophages and neutrophils). This, acute phase response in which neutrophils (PMN) predominate is followed by a chronic phase in which macrophages are the predominate cell type. A chronic inflammatory response implies the continued presence of the injurious agent, which may be represented even by a relatively inert biomedical polymer. Factors which can provoke increased levels of chronic inflammation include: extensive surgical injury; properties associated with the implanted polymer, including

TYPICAL CONSTITUENTS OF POLYMERS

BASIC CHEMICAL COMPOSITION
UNREACTED MONOMER, OLIGOMERS
CATALYSTS
ANTIOXIDANTS, STABILIZERS
FILLERS
PLASTICIZERS
SURFACE ACTIVE AGENTS
(lubricants, mould release agents)
EFFECTS OF MATERIAL PREPARATION
(processing, sterilization)
POLYMER MORPHOLOGY / TOPOGRAPHY
(smooth, rough, porous)

TABLE II

amorphous or semicrystalline. It will probably contain low molecular weight additives or impurities which may migrate to the interface such as plasticizers, stabilizers, specific modifying agents, possible degradation products, or have that have adsorbed on to the surface. In addition, the biomaterial may be rough or relatively smooth, porous, rigid or flexible. This is the "polymer surface" that will mediate the protein and cellular events at the interface.

physical irritation caused by the implant; bacterial infection; or host factors, such as poor blood supply or nutrition. During the chronic phase, fibrous connective tissue begins to form around the mass of leukocytes. Eventually, this process encapsulates the implanted polymer in a dense capsule of connective tissue, known as a foreign-body granuloma, the objective being to isolate the implant, and therefore its effect on the host, from the rest of the body. Accumulation of blood pigments, lipids and calcium salts is often observed within the granuloma and is indicative of a continuing inflammatory reaction.

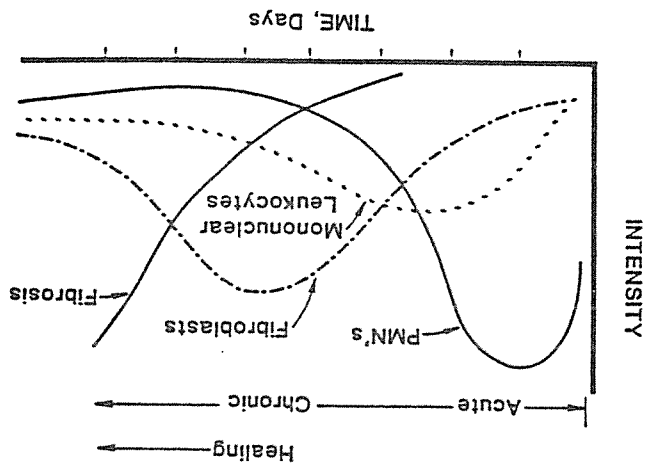


Figure 1. Schematic of the inflammatory and healing response to an implanted biomaterial. The intensity and duration of the reactions will vary according to the nature of the implant.

Macrophage-Polymer Interactions

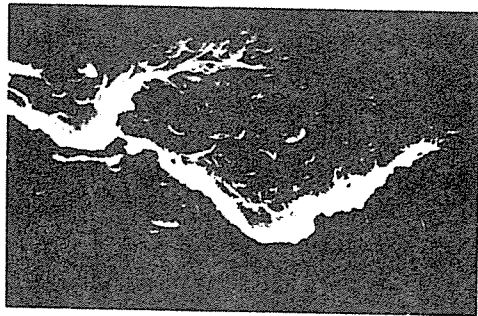


Figure 2. The macrophage-polymer interface.

Of particular interest with respect to biodegradation phenomena are the macrophage-polymer interactions (Figure 2). The observed accelerated biodegradation of biomedical polymers might be explained by the unique role of the macrophage. This cell is much longer-lived than the neutrophil, it is capable of synthesizing and releasing many

Oxygen in combination with mechanical stress or moderately elevated temperatures may also initiate the degradation (8). The reactions usually lead to the formation and decomposition of peroxides. The subsequent release of CO_2 and CO appear to be a common feature. In addition, degradation often leads to the inclusion of polar impurities into the polymer, that may act as initiating centers for further oxidation. The presence of strong oxidizing agents and other potentially harmful molecules at the polymer-macrophage interface indicates that the possibility of a cell-mediated oxidation mechanism for biomedical polymers. Consequently, the biostability of polymers that do not contain hydrolyzable bonds should not be assumed on the basis of there being no ultraviolet light or elevated temperatures in the body.

Enzyme-mediated hydrolysis

The possible involvement of hydrolytic lysosomal enzymes mediating biodegradation of biomedical polymers has been a popular hypothesis for the degradation of several biomedical polymers including polyesters, polyamides, polyolefins and segmented polyurethanes (6,12,21). However, while the effects of the enzymes have been clearly shown, no direct evidence for a particular mechanism between an enzyme and a solid synthetic polymer substrate has been demonstrated. Secretory products from macrophages, including lysosomal enzymes, do not necessarily provide a degradative environment *in vivo*. This was shown in a study in which a poly(etherurethaneurea) (PEUU) was subjected to an intense inflammatory reaction for an implantation period of three weeks (22). The intense host reaction was stimulated by the presence of an adjacent poly(vinyl chloride) implant containing an organotin cytotoxic stabilizer. Host reaction to the cytotoxic agent included very high concentrations of extracellular enzymes derived from lysed leukocytes. Nevertheless, no spectroscopic evidence for hydrolytic degradation was detected. This result was explained by the fact that most proteases have their optimum activity at low pH, often below 6.0. This poses little problem intracellularly, because the leukocytes can provide very low pH levels in the phagolysosomes without endangering the cell's viability. In the bulk exudate however the observed pH was >7.5

suggesting the enzymes have relatively low activity. More recently, this observation was confirmed by the studies of Zhao and Anderson (23). Strained PEU samples were subjected to the normal and cytotoxic-agent enhanced inflammatory reactions. In the latter environment, evidence for degradation was not detected, while the control conditions resulted in complete disintegration of the PEU (pelletthane 80A). These results highlight the importance of the interactions of viable macrophages with implanted polymers, and focuses our working hypothesis on the microenvironment at the macrophage-polymer interface. The pH in close proximity to the cell is likely to be quite low during a period of exocytosis and provides a model to explain our results and those of others who have shown that enzymes (under optimum pH conditions) can cause deterioration of polymer properties. Following this concept, we have proposed a possible mechanism for papain mediated hydrolysis of PEU (12), shown in Figure 3, which was adapted from the accepted mechanism for the hydrolysis of protein substrates (24).

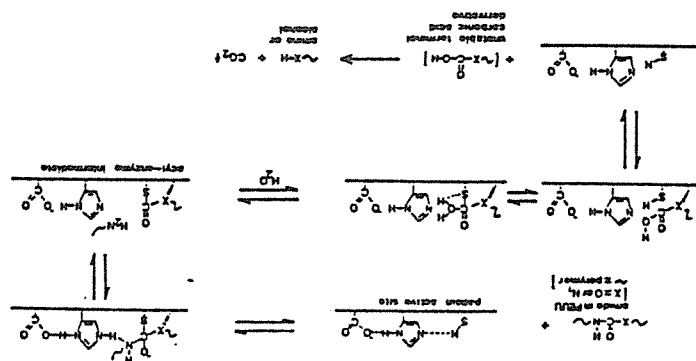


Figure 3. Papain-catalyzed hydrolysis of a poly(etherurethaneurea).

An important condition for the proposed mechanism depends on the ability of the enzyme to bind the polymer substrate, indicated in Figure 3 by the dashed line. Obviously no catalysis will occur without binding. Indeed the relatively long reaction times that are required to generate observable effects are suggestive of the binding being rate determining. Binding is normally achieved through non-specific hydrophobic interactions which suggests that the diphenyl group derived from MDI probably improves the enzyme affinity, while the substrate being in solid form markedly reduces the available binding sites.

Clearly, polymer surface composition in aqueous media and solvation effects of polar functional groups at the interface need to more precisely defined. Nevertheless, our studies and those of others are providing growing evidence for the occurrence of such an enzyme mediated hydrolysis reaction.

FUTURE PROGRESS

Progress in our understanding of biocompatibility and biodegradation phenomena will continue to rely heavily on the advances made in the underlying medical, science and engineering disciplines. Clinical retrieval programs will continue to provide important pathologic and statistical information on the performance on implanted prosthetic devices. In recent years, there have been significant advances in our knowledge of leukocyte function and leukocyte mediated reactions. On the materials side, our ability to accurately characterize a polymer surface is rapidly improving with refinements to the electron spectroscopy for chemical analysis technique as well as the increasing use of secondary ion mass spectroscopy. It seems reasonable to expect the application of these developments will yield important insights into biocompatibility and biodegradation within the next few years, particularly regard to the cell-polymer interface.

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POLYURETHANES AT THE BIOLOGICAL INTERFACE

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In an effort to probe the factors which affect the interaction between the surface of a multiphase polyurethane and blood, a series of butanediol-chain-extended polyetherurethanes was synthesized. These polyurethanes contained different levels of phase separation, produced by systematically varying the hard segment chemical structure by grafting ethyl and octadecyl groups or by incorporating propyl sulfonate groups. The bulk, surface, and canine *ex vivo* blood-contacting properties of these grafted polymers were evaluated using a variety of techniques including differential scanning calorimetry, dynamic mechanical analysis, tensile testing, x-ray photoelectron spectroscopy, contact angle measurements and animal experiments. It was determined that the alkyl chain length of the 10% alkylated polymers had a significant effect on the blood-contacting properties. The hydrophilicity and water uptake of the sulfonated polyurethanes increased with increasing ion content. Interestingly, the highly sulfonated polyurethanes had very low levels of platelet adhesion and activation accompanied by high levels of fibrinogen deposition.

Introduction

Polyurethane block copolymers have been proposed for use in blood-contacting applications due to a combination of their generally excellent physical properties and relatively good blood compatibility (1). It is desirable, however, to improve the blood compatibility of these materials to allow their use in demanding applications such as small-diameter vascular grafts, catheters, cardiac assist devices, and the artificial heart. Thrombus formation on polyurethanes and other blood-contacting biomaterials can lead to occlusion of vascular grafts or catheters, while the detachment (embolization) of these thrombi may result in tissue damage or strokes. Several strategies have been proposed to achieve blood compatibility in the design of blood-contacting materials. One method (2) involves the synthesis of a highly hydrophilic, mobile interface that appears "bland" to blood components. These materials have been hypothesized to have a reduced tendency for protein adsorption and platelet adhesion. Another method involves the introduction of highly hydrophobic groups to the blood-contacting interface, either by using a highly hydrophobic polymer such as silicone rubber or by grafting long alkyl chains to a relatively hydrophilic material such as a polyurethane block copolymer.

By using long-chain alkyl grafting methods, Eberhart and coworkers (3) have improved the blood compatibility of polyurethane block copolymers, and similar strategies have resulted in the reduction of platelet and fibrinogen deposition in canine *ex vivo* blood-contacting experiments (4). Another method involves incorporating ionic functionality into the polymer. This strategy could also result in a highly hydrophilic and mobile interface resulting in a low driving force for protein adsorption and cell adhesion. Previous studies of the role of ionic interactions have been reviewed (5,6). In the published literature, it is seen that ionic groups play an important, but incompletely understood, role of blood-material interactions. This work was stimulated in part by recent studies of "heparinoid" materials that have generally involved the synthesis of macromolecules with sulfonate, carboxylate, or other functional groups. A number of investigations have indicated that the presence of specific ionic groups plays a role in either anticoagulant action or platelet reactivity (7-17). In some of these studies, the action of sulfonate and/or carboxylate groups has been specifically studied and related to anticoagulant activity.

One of the first investigations of the biological interactions of ionic polyurethanes was reported by Rembaum et al. (18). In their study, polyether polyurethanes containing positive charges in the backbone (cationomers) were synthesized by ionene hard segments by the reaction of ditertiary amines with dihalides. Because these polymers were subsequently reacted with heparin to yield polyurethane-heparin complexes, it is difficult to determine if the ionic character of the polyurethane itself contributed to the biological response. In another study, Ito et al. (19) examined anionic polyurethanes with carboxylic functionality. These investigators found that the anionic polyurethane selectively adsorbed albumin, did not cause a conformational change of plasma proteins adsorbed, suppressed the adherence and deformation of platelets, but did not deactivate the clotting system and were thus moderately thrombogenic. A heparin-bound derivative of this anionic polyurethane was not favourable for albumin adsorption, caused plasma protein denaturation, induced platelet adherence and activation, but did not activate the clotting system (as measured by thrombin times). The question of "biocompatibility" was not resolved for these materials, as one would presume that neither platelet activation nor activation of the clotting system would be desirable. Two separate studies by Cooper and coworkers (6,20) showed that ionization of polyurethanes may be a useful technique to improve blood compatibility. In the first investigation, two uncharged polyurethanes based on methylene bis (p-phenyl isocyanate) (MDI), N-methyldiethanolamine (MDEA), poly(tetramethylene oxide) (PTMO) of MW 1000 containing 21.5 and 38 weight percent MDI were examined using a canine *ex vivo* blood-contacting experiment. Also studied were

the sulfonate-containing zwitterionic, neutralized anionic, and quaternized cationic derivatives of the MDEA-chain-extended base material containing 38 weight percent MDI. The polyurethane zwitterionomer and anionomer showed lower levels of platelet and fibrinogen deposition than the uncharged polyurethane, while the polyurethane cationomer showed relatively high level of thrombus deposition. In a similar polyurethane system containing 24 weight percent MDI, the zwitterionomer exhibited less thrombus deposition than the non-ionic base material. The exact mechanism of this action, however, was not investigated. A base polymer based on MDI, PTMO of molecular weight 1000 and 1,4-butanediol (BD) was chosen for this investigation. The use of butanediol as a chain extender results in a polyurethane (PEU) with superior physical properties than observed with an analogous polymer chain-extended with MDEA, due to the superior ability of the hard segments in the BD-chain extended system to aggregate and crystallize (1). The use of butanediol is also more common industrially, and BD is the chain extender used in medical grade Pellethane (1) and Vialon (21) polyurethanes. Thus, determination of the feasibility of derivatization strategies for industrially important polyurethanes is of interest and is another goal of this study.

In this study, polymer modifications in solution were performed, and after precipitation and processing, bulk characterization techniques were used to study physical properties. The bulk properties were related to surface properties, and these polyurethanes were evaluated for blood compatibility in a canine *ex vivo* blood contacting experiment. Although bulk modifications were performed, there is no reason why these polymers cannot be used as coatings or be adapted as a surface modification.

Experimental

The base PEU contained 48% hard segment consisting of 4,4'-diphenylmethane diisocyanate (MDI), 1,4-butanediol (BD), and poly(tetramethylene oxide) (PTMO, MW=1000) as the soft segment. A biomolecular nucleophilic displacement reaction was used to replace 10% of the urethane hydrogens with C18 and C2 alkyl groups and the extent of sulfonate substitution was systematically varied from 5 to 20 percent replacement of the urethane hydrogen atoms.

After synthesis the, derivatized polymers, as well as the base PEU polymer, were extracted using toluene for approximately 48 hours in a Soxhlet extractor in an effort to remove contaminants, oligomeric species, and residual propane sulfone if present. After briefly drying under vacuum to remove residual toluene, the resulting polymers were dissolved in dimethylacetamide (DMAc) and then cast into teflon pans to make films for bulk property testing or coated on the inner lumen of chromic acid-oxidized polyethylene tubing for surface property and blood compatibility evaluation (20,22).

In both casting procedures, cast films were first dried under a nitrogen stream (tubing) or in a 70 degree Centigrade forced-air oven (bulk sheets) for at least 48 hours to remove most of the DMAc. The final drying stage involved drying of the coated tube or sheet under vacuum at 60 degrees Centigrade for at least 48 hours to remove residual DMAc.

The bulk and surface properties the polyurethanes were characterized using: differential scanning calorimetry (DSC), transmission infrared spectroscopy (FTIR), dynamic mechanical analysis (DMA), tensile testing, electron spectroscopy for chemical analysis (ESCA) and underwater static contact angle measurements which have been previously described (4,5). A canine *ex vivo* series shunt experiment was used to evaluate the blood-contacting properties of the polymers (22). A diagram of the animal experiment showing the shunt, catheter, and flow measuring and recording instrumentation is shown in Figure 1.

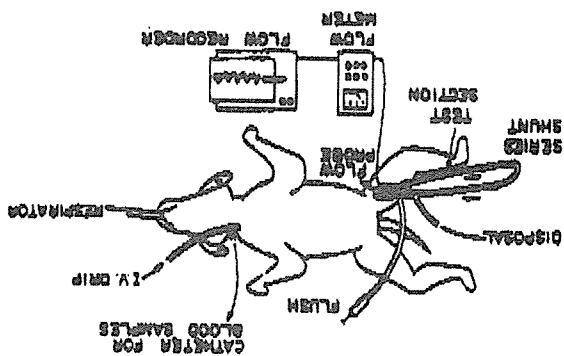


Figure 1

Results and Discussion

C2 groups were grafted onto the polyurethane to distinguish between the effect of phase mixing and the C18-albumin interaction. DSC and DMA verified the enhanced phase mixed morphology of the alkyl derivatized polymers. ESCA and contact angle analysis indicated that alkylation increased the amount of aliphatic carbon at the vacuum interface and also enhanced the hydrophobic nature of the polymer at the water interface.

The canine *ex vivo* results showed that the PEU-C2-0.1 was much more thrombogenic than the base PEU and PEU-C18-0.1, indicating phase separation was an important parameter in the blood-contacting properties of these polymers. In addition, these results suggest that PEU-C18-0.1 suppressed the slightly more thrombogenic nature of the base PEU.

For the hydrophilic sulfonated polyurethanes DSC and DMA indicated that low levels of ion incorporation (5-15%) decreased the degree of phase separation due to the disruption of hard segment packing. However,

PEU-S03-0.2 had increased phase separation possibly due to interaction of the N-H groups with the sulfonate groups and the enhanced polarity different between the hard and soft segments. The polymer's water absorption was significantly enhanced with increasing ionic content varying from 4% for PEU-S03-0.05 to >1000% for PEU-S03-0.2. Surface characterization indicated that the polymer-air interface was mobile and capable of rearranging to minimize the interfacial energy at a water or vacuum interface.

The blood-contacting studies indicated that the highly sulfonated polyurethanes had very low platelet deposition and activation for blood exposure times up to 7 days, yet they adsorb large quantities of fibrinogen. Further studies investigating the mechanism by which the sulfonate groups inhibit platelet activation in the presence of high concentrations of fibrinogen are under way.

These studies demonstrate the complex interrelation between a polyurethane's morphology, and its bulk, surface and blood-contacting properties.

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ANTI-TUMOR ACTIVITY OF POLYANIONS FROM COPOLYMERS RELATED TO DIVEMA

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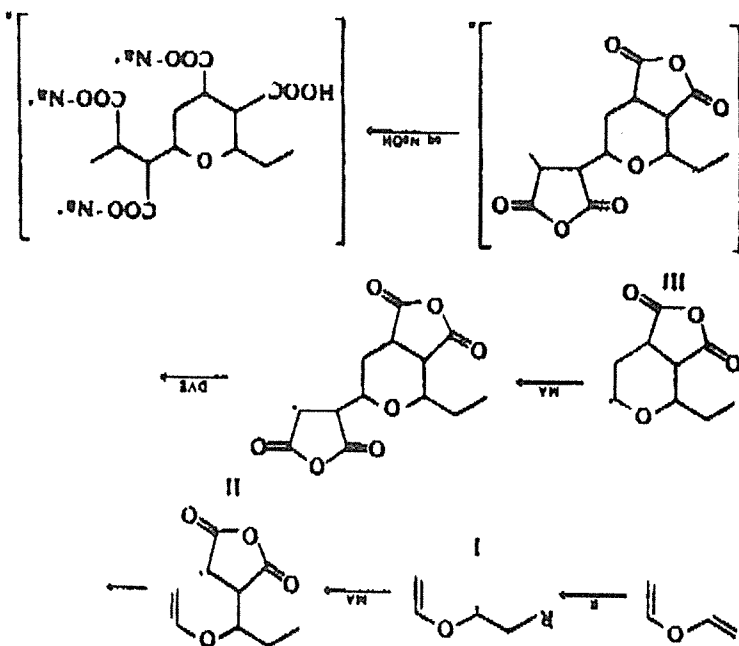
During development of the theory of cyclopolymerization an alternating cyclocopolymerization between a non-conjugated donor diene and an acceptor alkene was discovered. The first example was copolymerization of divinyl ether (DVE) with maleic anhydride (MA) in a 1:2 molar ratio, now known as DIVEMA. This polyanhydride is readily hydrolyzed to the corresponding carboxylic acid. It had been shown that the ethylene-maleic anhydride copolymer, as its polycarboxylic acid, exhibited significant anti-tumor activity. Studies led to observations that DIVEMA also possessed significant anti-tumor properties. The results are presented in another paper at this symposium.

This paper deals with a synthetic effort to produce new copolymers related to DIVEMA and their evaluation as anti-tumor agents. The most significant of these copolymers are: 1,4-pentadiene:MA, DVE:itaconic anhydride, DVE:citraconic anhydride, divinylidimethylsilane:MA, and furan:MA. These copolymers were fractionated to yield fractions of controlled molecular weight (MW) and molecular weight distribution (MWD) in efforts to establish the relationship of the polymer properties and their anti-tumor activity.

ANTI-TUMOR ACTIVITY OF POLYANIONS FROM COPOLYMERS RELATED TO DIVEMA

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A variety of synthetic polyanions are available, and their physiological properties have been compared with naturally occurring polyanions. Probably the most widely investigated synthetic polyanion is the 1:2 regularly alternating cyclo-copolymer (DIVEMA) of divinyl ether (DVE) and maleic anhydride (MA). (Eq. 1) This material has been investigated extensively, both from the standpoint of its chemical structure² and its anti-tumor activity.³ It has been shown⁴ to possess a wide spectrum of biological activity⁵ and is an interferon inducer.⁶ A particularly effective variation (MVE-2) will be discussed in another lecture at this symposium.⁷



The broad spectrum of biological activity of DIVEMA and/or the modified form, MVE-2, has been reemphasized by the results of some recent studies which are reported here. These novel results also imply that the structurally related copolymers reported in this paper may possess equivalent or enhanced efficacy in some of these applications.

in a recent study on therapy of peritoneal murine cancer (Rencana) with biological response modifiers (BRM), both MVE-2 and interleukin-2 were used.⁶ While both significantly increased the survival time of tumor-bearing mice, only MVE-2 led to definite cures. A single injection of MVE-2 cured 20% of the tumor-bearing mice, while repeated administration of the drug at 12 day intervals cured 70% of the mice. Combined therapy with doxorubicin hydrochloride and a single dose of MVE-2 cured 90% of the tumor-bearing animals. This superior therapeutic efficiency of MVE-2 compared to that of interleukin-2 may be due to its ability, after inoculation, to generate and maintain high levels of cytotoxic effector cell activity for an elevated period of time within the peritoneal cell population. Additionally, MVE-2 augments effector cell activity in the liver, lungs, spleen, and blood and may therefore more efficiently interfere with metastasis formation in those compartments. The additive effects of MVE-2 and the chemotherapeutic agent suggest that more effective therapy may be achieved by the combination of immunotherapy with BRMs with chemotherapeutic drugs.

A more recent study dealing with interleukin-2 has produced significant and interesting results.⁷ The adoptive transfer of tumor-infiltrating lymphocytes (TIL) expanded in interleukin-2 (IL-2) to mice bearing micrometastases from various types of tumors showed that TIL are 50 to 100 times more effective in their therapeutic potency than are lymphokine-activated killer (LAK) cells. Therefore the use of TIL was explored for the treatment of mice with large pulmonary and hepatic metastatic tumors that do not respond to LAK cell therapy. Although treatment of animals with TIL alone or cyclophosphamide alone had little impact, these two modalities together mediated the elimination of large metastatic cancer deposits in the liver and lung. The combination of TIL and cyclophosphamide was further potentiated by the simultaneous administration of IL-2. With the combination of cyclophosphamide, TIL, and IL-2, 100% of mice ($n = 12$) bearing the MC-38 colon adenocarcinoma were cured of advanced pulmonary metastases. Techniques have been developed to isolate TIL from human tumors. These experiments provide a rationale for the use of TIL in the treatment of humans with advanced cancer. In view of the earlier work in which MVE-2 was shown to be superior to interleukin-2, it follows that MVE-2 as well as other structurally related materials should be studied with

tumor-infiltrating lymphocytes plus cyclophosphamide, as reported above using interleukin-2.

The mode of administration of choice by scientists conducting animal studies with DIVEMA and the modified MVE-2 has been by intraperitoneal (i.p.) injection. A recent study in support of the clinical studies which have been underway has shown that i.p. administration of MVE-2 in dogs gave lower renal toxicity effects than by intravenous (i.v.) administrations.⁸

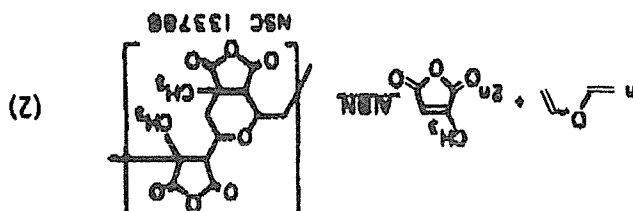
Alternate routes of administration of MVE-2 [27100-68-1] (mean mol. wt. of 11,000) were studied which may be less toxic and more efficacious than the present i.v. route of administration. Renal toxicity and proteinuria were observed after i.v. administration of MVE-2 (25 mg/kg) to dogs twice a week for up to one month. In contrast, when MVE-2 was given i.p., there was no evidence of renal toxicity. Furthermore, macrophase accumulation in the mesenteric lymph nodes, mediastinal lymph nodes and the peritoneal cavity were increased. Tissue concentrations of ¹⁴C in mesenteric lymph nodes and thymus were much higher after an i.p. than after an i.v. dose of ¹⁴C MVE-2. Remarkably high concentrations of ¹⁴C were also observed in the mediastinal lymph nodes following an i.p. dose. These distribution studies indicated that a portion of the drug administered i.p. was absorbed directly into the lymphatic system draining the peritoneal cavity. Furthermore, i.p. administration may provide direct stimulation of peritoneal macrophages and also modulate immune function through direct effects on lymph nodes trapping the drug.

Many attempts to modify DIVEMA to enhance its effectiveness as well as reduce its toxicity have been made. Among these is a recent patent which describes a procedure for administration of DIVEMA in the form of its partial calcium salt. DIVEMA polymer Ca salts [68527-96-8] (mol. wt. 2000-100,000), degree of neutralization 5-70%) were found to be as effective as the corresponding alkali metal salts in treating tumors or viruses, and are much less toxic. The toxicity (mortality to mice in 14 days) of these Ca salts was 0/10 at 750 mg/kg body weight, compared with 9/10 at 90 mg/kg for the Na salt. The average increase in survival time for mice with Colon 26 tumors treated with 12.5 mg/kg 40%-neutralized Ca salt was 210%, compared with 218% for the Na salt.

The 1:1 copolymer of furan and MA, designated as NSC 119166, and its half-amide, half-ammonium salt, designated as NSC 119167, as

In an extension of the work on DVE copolymerizations, Butler, Badgett, and Sharabash investigated the copolymerization of furan and MA. It was shown that copolymerization occurred readily, that the rate of copolymerization was maximum at monomer mole fractions of 0.5, and that 1:1 alternating copolymers were obtained. The structure of the copolymer, ¹³C as shown by ¹³C NMR studies, was assumed to involve 1,4-addition by furan and 1,2-addition by MA. Further experiments also showed that the Diels-Alder adduct could be converted to a copolymer of the same structure under the same conditions that the monomer pair could be copolymerized. These results suggest that a common intermediate is formed in both the Diels-Alder adduct formation and the copolymerization.

12 Copolymer of Divinyl Ether and Citraconic Anhydride



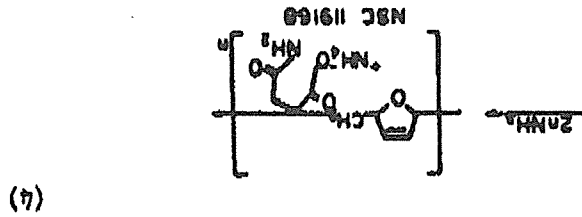
Anti-tumor activity of varying degrees appears to be a general property of copolymers related in structure to the DIVEA copolymer. The corresponding copolymer of DVE and citraconic anhydride (CA), NSC 133788, as shown in Eq. 2, has been evaluated, the results of which gave mean survival times (6/6) of T/C (%) = 130 at the optimum dose. More recent results based on fractionated samples of known molecular weight and molecular weight distribution are shown in Table 1.

The anti-tumor activity of a number of structures closely related to DIVEA were recently discussed by Butler. These copolymers were prepared in a manner similar to that reported earlier for the synthesis of DIVEA.¹²

Many structural modifications of DIVEA have been synthesized which also possess anti-tumor activity and interferon-inducing capability.¹⁰ This lecture will review the synthesis and biological evaluation of these materials.

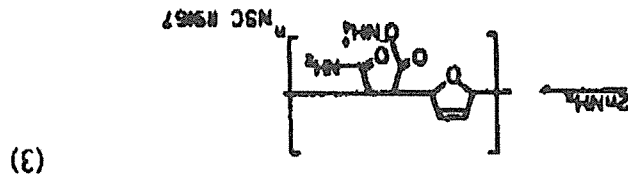
This copolymer has now been fractionated and samples of known molecular weight and molecular weight distribution have been evaluated for their anti-tumor properties. The results are shown in Table I.

Half-Amide, Half-Ammonium Salt of 1:1 Copolymer of Furan and Itaconic Anhydride

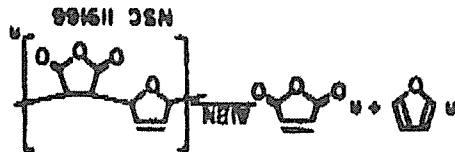


In addition, the copolymer of furan and itaconic anhydride (1A), designated as NSC 119165, and its half-amide, half-ammonium salt, designated as NSC 119168, were synthesized³ as shown in Eq. 4, and the copolymer derivative was evaluated. NSC 119168 gave survival times (6/6) of T/C (%) = 150 at the optimum dosage level.

Half Amide, Half Ammonium Salt of 1:1 Copolymer of Furan and Maleic Anhydride



1:1 Copolymer of Furan and Maleic Anhydride



shown in Eq. 3, were evaluated for their anti-tumor activity against mouse L-1210 lymphoid leukemia. Evaluation of NSC 119166 gave survival time (6/6) to T/C (%) = 130 at the optimum dose. NSC 119167 gave survival time (6/6) of T/C (%) = 125 at the optimum dose. More recent results, based on fractionated samples of known molecular weight and molecular weight distribution are shown in Table I.

Other 1,4-dienes, e.g., divinylidialkylsilanes, (DVS) 1,4-pentadiene (PD), and divinyl sulfone (DVS) can be substituted for DVE in copolymerization with MA. Results of anti-tumor evaluations of these copolymers are shown in Table 1.

A variety of maleimides have been copolymerized with DVE to yield copolymers, many of which have shown effective anti-tumor activity. Among the maleimides studied were maleoyl-d,l-leucine (N-d,l-L) maleoyl-l-phenylalanine (N-l-PA), N-morpholinomethylmaleimide (N-MM), N-ethylmaleimide (N-EM), N-carboethoxymaleimide, maleoyl-d,l-phenylalanine, maleoyl-d,l-alanine, and maleoylglycine.⁵ These copolymers had inherent viscosities in the range of 0.19-0.66 dL/g and number-average molecular weights of 26,000-170,000 (determined by GPC on their methyl esters). The results of anti-tumor evaluation of certain of these copolymers are shown in Table 1.

Copolymers of DVE, 2-chloroethyl vinyl ether (CEVE) and styrene (St) with MA have been derivatized with 5-fluorouracil (5-FU).

Umrigar, Ohashi, and Butler¹⁶ copolymerized DVE, styrene, and 2-chloroethyl vinyl ether (CEVE) (CMAFU) to yield copolymers of molecular weights in the range of 5000-7500. The styrene (St) and CEVE copolymers were shown by analysis to be 1:1 alternating copolymers, while the DVE copolymer

TABLE I

Results of Anti-tumor Evaluation^a

Copolymer	Tumor ^b	Dose mg/kg	T/C ^c %
DVE-CA	PS	6	267
DVE-N-d,l-L	PS	25	126
DVS1-MA	PS	25	123
PD-MA	PS	50	121
FU-MA	PS	50	147
FU-MA ^d	PS	50	147
FU-1A ^d	PS	100	147
DVE-MA ^e	PS	100	174
EVE-MA ^e	PS	100	180
St-MA ^e	PS	50	152

^a Based on "Instruction 14, National Cancer Institute" program; ^b PS = P388 lymphocytic leukemia; LE = L-1210 lymphoid leukemia; ^c Ratio of mean survival times of treated (T) to control mice (C) in percent; ^d Half amides; ^e 5-FU deriv.

was 2:1 molar in CMAFU:DVE content. The results of the evaluation of these copolymers by the Development Therapeutics Program, Division of Cancer Treatment, National Cancer Institute are shown in Table 2.

5-Fluorouracil was released from these copolymers hydrolytically, and their respective rates of release were determined from dispersions in 0.5 M NaCl. Under these conditions, the monomer (CMAFU) released 5-FU almost instantly and completely. On the other hand, the St:CMAFU and CEVE:CMAFU copolymers were resistant to hydrolysis and released 5-FU gradually. DVE:CMAFU was hydrolyzed faster than the other two copolymers, but much slower than the monomer. The hydrophobic character of the St:CMAFU copolymer appears to be important for slow release of 5-FU.

The copolymers have now been fractionated and samples of known molecular weight and molecular weight distribution have been re-evaluated for their anti-tumor activity. The results of this test are shown in Table 1.

DIVEMA has been modified¹⁸ by covalently bonding methotrexate, N-[4-methyl-2,4-diamino-6-pyridinyl-methylamino)-benzoyl] glutamic acid, a widely used anti-tumor agent and a folic acid antagonist, to several copolymers of various molecular weights. This copolymer was selected as a potential carrier for this agent because of its established anti-tumor and immune-stimulating activity.

TABLE 2
Results of Evaluation of Polymers by Development Therapeutics Program, Division of Cancer Treatment, National Cancer Institute

T/C (%)		Dose ^a (mg/kg)	
		St:CMAFU (NSC 255081)	CEVE:CMAFU (NSC 255082)
		DVE:CMAFU (NSC 255083)	
400	94	129	112
200	192	165	189
100	171	141	172
50	133	127	140
25	115	117	125
			105

^aDose in mg/kg of animal weight via intraperitoneal injection in CD₂F₁ male mice, 6 of 6 survivors after fifth day against P388 lymphocytic leukemia; ratio of test animal (T) to control (C) survival times, in percent.

Copolymers having initial M_n values ranging from 4,400 to 24,000 were used. Using molar ratios of the DIVEA repeating unit of the copolymer to methotrexate ranging from 1.9 to 3.5, and varying the reaction time from 24 to 96 h, methotrexate residues ranging from 3.0 to 42.6 mol % were introduced. Linkage to the polymer occurred at the 2- or 4-amino groups of the pteridine ring.

A methotrexate-substituted derivative of DIVEA was evaluated (NIH screening test No. NSC 282447) for dihydrofolate reductase inhibitory activity in vitro and anti-tumor activity in vivo. The preliminary pharmacological studies showed inhibition of dihydrofolate reductase and cytotoxicity to L-1210 lymphoid leukemia cells in vitro, similar to free methotrexate.

Recent studies report on the synthesis of derivatives of DIVEA with adriamycin (ADR) and daunomycin (DM) and the anti-tumor evaluation of these derivatives.¹⁹ The compounds were synthesized by reaction of the drugs with DIVEA. The content of ADR moieties in the DIVEA could be varied depending on the reaction conditions up to 35.8 wt. %. Considering the low toxicity and the high possibility of renal excretion, DIVEA with M_w of 7000 and $M_w/M_n = 1.6$ was used for the derivatization. The rate of drug release from the derivative was determined under physiological conditions by reversed phase HPLC. Within 14 days only 15% of the attached ADR was released from the ADR derivative. The anti-tumor activity of the derivatives was tested in vitro and in vivo against mouse P388 leukemia. The ADR derivative proved to be 28 times less active than ADR in vitro, which could be explained from the slow drug-release. On the contrary 50% of the leukemic mice treated by this derivative survived more than 60 days, whereas no mice given ADR alone or the admixture of the ADR and DIVEA survived 30 days. An anti-tumor activity of the polymeric derivative better than that of the free drug was also observed in vivo with DM. Such a polymeric effect can be attributed either to the change in body distribution, the difference in pharmacokinetics, or the slow drug release.

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CONTROLLED DRUG RELEASE FROM BIOERODIBLE IMPLANTS - FACTORS THAT
AFFECT RATE OF POLYMER BIOEROSION

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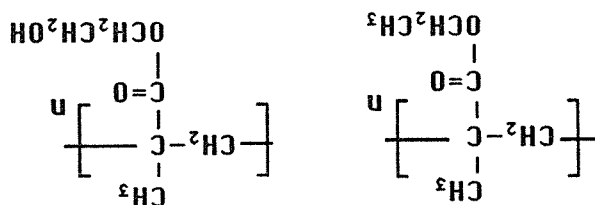
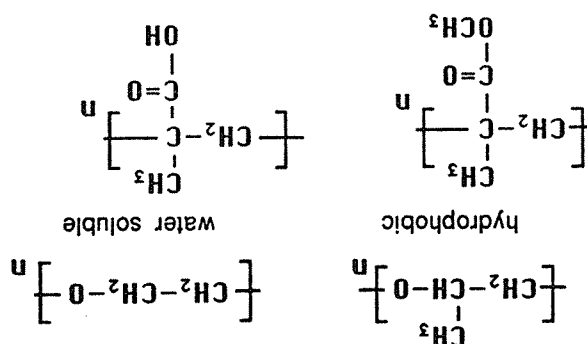
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This article will discuss factors that are important in determining degradability of polymers. Even though the degradation of consumer-type products is for ecological reasons clearly important, this article will only focus on the degradation of polymers which are currently under development as implants in human or animal tissues. Biodegradation of synthetic, hydrophobic polymers can occur by two distinct mechanisms; chemical hydrolysis in the aqueous environment within tissues or enzymatic hydrolysis. Of these, for reasons which will be discussed later, chemical hydrolysis is by far the most important mode of degradation. In attempting to understand polymer hydrolysis, the following factors must be considered:

1. Polymer Hydrophilicity
2. Chemical Reactivity of Labile Linkages
3. Autocatalysis
4. Chain Flexibility

Because polymer hydrolysis requires access of water to the labile linkage, the ability of polymers to absorb water is clearly important. A number of factors can affect polymer hydrophilicity, but the three most important ones are chemical structure of the polymer, its glass transition temperature and its degree of crystallinity.

a. **Chemical structure of polymer** - polymer hydrophilicity is determined by the nature of substituents on the polymer chain, and relatively small structural changes can have a major effect and convert a hydrophobic, water insoluble polymer into a water soluble polymer. Some examples are shown below:



b. **Glass transition temperature** - the glass transition temperature (T_g) of a polymer is a narrow temperature range below which the polymer is in a glassy state where intermolecular forces are such that segmental motion of chains is not possible and above which the thermal energy supplied to the polymer is able to overcome these forces and

segmental motion of polymer chains becomes possible. The ability of polymer chain segments to move relative to each other has a profound effect on diffusion of molecules through the polymer because diffusion occurs by movement of molecules between polymer chains. Then, because rate of diffusion of water through glassy polymers is extremely slow, rate of hydrolysis of polymers that at the body temperature of 37°C are below T_g will also be slow even for chemical linkages which are quite hydrolytically labile. However, for polymers that at 37°C are above their T_g , rate of hydrolysis will proceed at rates largely determined by the reactivity of the hydrolytically labile linkages.

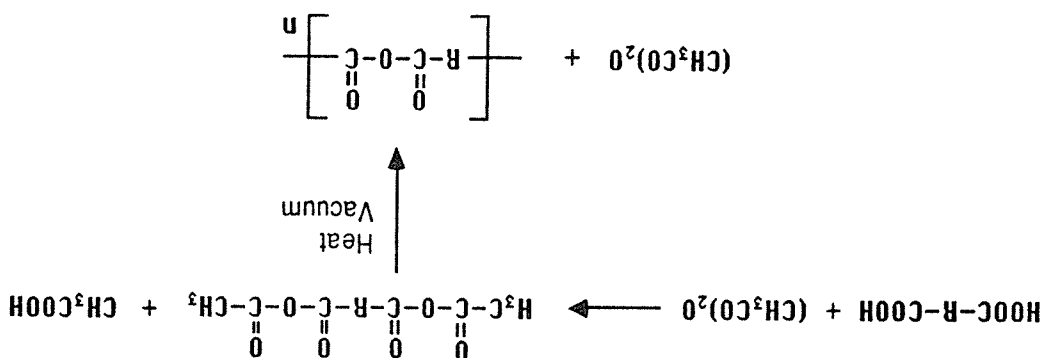
c. Crystallinity - polymers that have regular structures are able to organize their chains into crystalline regions. However, unlike small organic molecules, most polymers form materials which are comprised of both amorphous and crystalline regions and percent crystallinity refers to the amount of crystalline regions relative to the amorphous regions.

Except for materials which are very highly crystalline, a useful model is the fringed micelle model, depicted in Figure 1. In this model, polymer chains wind in and out of crystalline and amorphous regions.

Because polymer chains in crystalline regions are rigidly held together by strong forces they are virtually impenetrable to water and even very hydrolytically labile linkages in the crystalline regions are very unreactive. Thus, percent crystallinity will have a significant effect on rate of polymer hydrolysis which will decrease as percent crystallinity increases.

A number of systems under development in controlled drug release applications take advantage of these factors to control rate of bioerosion. These will be illustrated with polyanhydrides and polyesters.

Polyanhydrides. These were first investigated by Carothers [1] in 1932 as potential textile fibers and were later also investigated by Conix [2] and by Yoda [3]. They are currently under active development by Langer and coworkers [4,5] as a bioerodible drug delivery system. Polyanhydrides are best prepared as shown in Scheme 1 [6].



SCHEME 1

The effect of glass transition temperature, and crystallinity on hydrolysis rates of polyanhydrides is shown in Figure 2 [2]. The data show that the aliphatic poly (sebacic anhydride) (I) hydrolyzes at very rapid rates because it is a noncrystalline material which is above its glass transition temperature at the hydrolysis temperature. Hydrolysis rate of polymer II is significantly retarded because this amorphous polyanhydride is in its glassy state at the hydrolysis temperature. Polymer III degrades at extremely slow rates because it is a highly crystalline material. In fact, its hydrolysis rate is close to the crystalline polyester IV which contains very much less reactive ester linkages in the polymer backbone. This principle has been used in the design of biodegradable drug delivery systems and erosion rates measured as percent weight loss for various polyanhydrides is shown in Figure 3 [4]. In these measurements thin disks of the polymer were exposed to a pH 7.4 buffer and weight loss followed as a function of time. The data show a pronounced effect of polymer composition on rate of weight loss. Further, weight loss is linear with time which indicates that the disks are undergoing surface erosion.

When a drug is incorporated into the polymer and the disks placed in a pH 7.4 buffer, results shown in Figure 4 were obtained [4]. After an induction period, drug delivery and concomitant polymer erosion is observed up to 9 months, where the experiment was discontinued. The concomitant erosion and drug release again indicates that surface erosion occurs.

Aliphatic Polyesters. Polyesters based on lactic and glycolic acid have occupied a preeminent position among biodegradable polymers for many years because they degrade to the natural metabolites lactic and glycolic acid [7,8]. Because lactic acid has a chiral center, it can exist as the D and L enantiomers or as the DL racemic form. Because poly-D or poly-L lactic acid forms a stereoregular polymer that is able to crystallize while the nonstereoregular racemic DL form can not, erosion rate of the DL form is much more rapid. The ability of poly(L-lactic acid) to crystallize can be modified by copolymerization with another monomer such as glycolic acid and erosion rates of various such copolymers are shown in Figure 5 [9]. In this particular case, changes in erosion rates are due to changes in crystallinity and also in hydrophilicity because glycolic acid is more hydrophilic than lactic acid.

An interesting relationship between polymer degradation and degree of crystallinity is shown in Figure 6 which shows that degree of crystallinity increases with time [10]. This behavior is typically observed in the hydrolysis of semicrystalline polymers and is due to chain cleavage reactions in the amorphous regions. As a consequence of these chain cleavage reactions, chain mobility in the amorphous regions increases and additional crystallization can take place. The increased crystallinity will have a retarding effect on polymer bioerosion.

2. CHEMICAL REACTIVITY OF LABILE LINKAGES

Clearly, the rate at which bonds in a labile polymer cleave will have a very significant effect on the rate at which the matrix hydrolyzes. Reactivity of linkages in a hydrolytically labile polymers can be manipulated by either changing their chemical nature or by changing the pH within the environment surrounding a pH-sensitive polymer.

a. Changes in chemical structure - although this approach has not been used extensively in controlled release applications, it has been used in the development of bioerodible hydrogels for protein delivery. In preparing such materials, an unsaturated water-soluble polyester is prepared by the condensation of fumaric acid and a low molecular weight poly(ethylene glycol) and this material is then crosslinked by free radical copolymerization with a water-soluble monomer such as N-vinyl pyrrolidone [11]. Because all components are water-soluble, polypeptides can be immobilized in the hydrogel by first

preparing a solution containing all components and then crosslinking the linear polymer using redox initiation. This process is shown in Scheme 2.

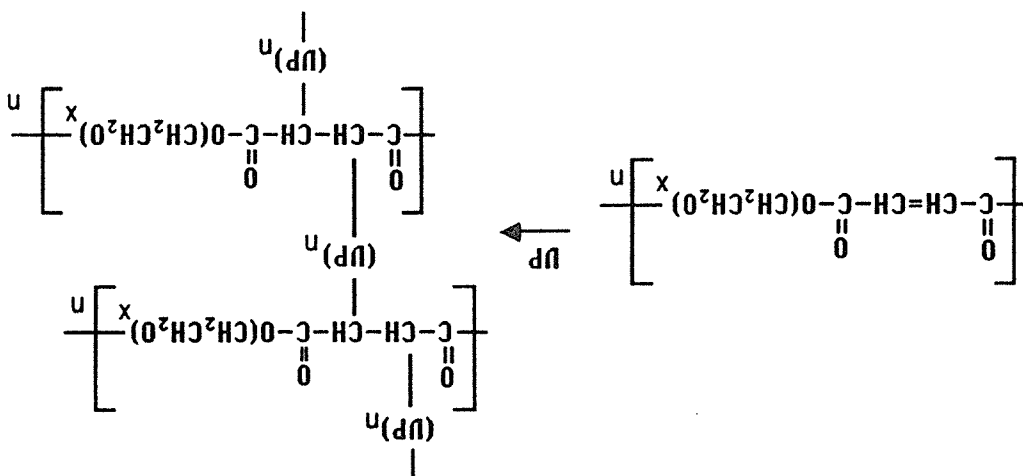
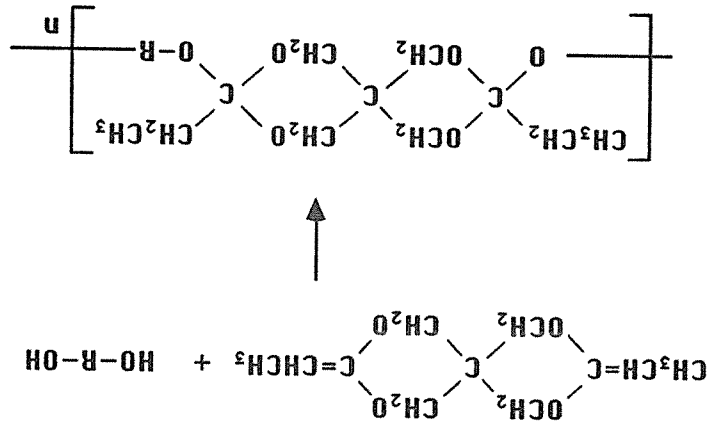


Figure 7 shows release of bovine serum albumin (BSA) immobilized in the polyester hydrogel prepared with 40 mole% N-vinyl pyrrolidone [11]. As expected, rate of BSA release is slow because rate of hydrolysis of aliphatic polyesters at pH 7.4 and 37°C is slow. However, rate of hydrolysis of ester groups can be significantly accelerated by placing an electron-withdrawing substituent vicinal to the ester groups because such groups can stabilize an electron-rich transition state. A particularly effective group is a carbonyl group and the rate of hydrolysis of α -keto ethyl propionate relative to methyl acetate is enhanced by a factor of 10^4 . Thus, when polyester hydrogels are prepared from fumaric acid, poly(ethylene glycol) and activated diacids such as diglycolic, ketomalonic or ketoglutaric, release of BSA is greatly accelerated as shown in Figure 8 [11]. Further, rate of BSA release corresponds to the expected rate of ester hydrolysis verifying that release is indeed erosion controlled.

b. Changes in the pH environment surrounding a polymer - the chemical reactivity of a pH sensitive polymer can also be manipulated by varying the pH within the environment surrounding the polymer.

When a drug and a latent acid catalyst such as maleic anhydride are mixed into the polymer and thin disks fabricated, erosion rate and concomitant release of the incorporated drug can be controlled by varying the concentration of anhydride incorporated into the polymer or by varying its pKa. This is shown in Figure 10 [14]. Further, as shown in Figure 11, weight loss of polymer and release of the incorporated drug and excipient occur concomitantly.

SCHEME 3

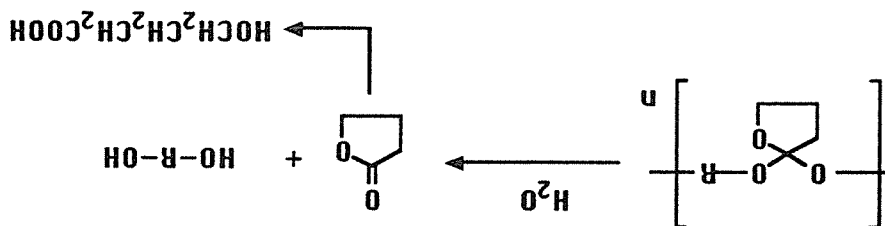


This approach has been successfully used with the pH-sensitive poly (ortho ester) systems by incorporating an acidic excipient into the polymer matrix [12]. The erosion process is schematically depicted in Figure 9. As shown, water permeates the polymer matrix, dissolves the acidic excipient and as a result of the lowered pH, polymer erosion in the surface layers accelerates. As a consequence of this process an eroding front develops and moves inward into the device. Because polymer hydrolysis in the eroding layer results in polymer solubilization drug and excipient immobilized in the polymer is released to the outside environment. Further, provided that surface geometry does not change significantly during the lifetime of the device, constant, or zero order kinetics are observed. Poly(ortho ester) can be prepared by the condensation of a ketene acetal and a polyol as shown in Scheme 3 [13].

3. AUTOCATALYSIS

Autocatalysis is observed when polymer degradation products catalyze rate of polymer hydrolysis. In controlled release applications this is not a desirable feature because rate of polymer hydrolysis will accelerate as the hydrolysis proceeds so that release of incorporated therapeutic agents will also accelerate.

One example of such an autocatalytic process is the hydrolysis of poly(ortho esters) prepared by the reaction between diethoxy tetrahydrofuran and diols [15].



Because, as previously shown, polymers containing ortho ester linkages are acid-sensitive, the hydroxybutyric acid degradation product acts as a catalyst which in the absence of stabilizing additives leads to an uncontrolled rate of polymer erosion. However, when basic excipients such as Na_2CO_3 are added to the polymer, the liberated hydroxybutyric acid is neutralized and control over erosion rate can be achieved [16].

Another example of autocatalysis is the hydrolysis of polyesters where the liberated carboxylic acid also acts as a catalyst for the hydrolysis process [10].

4. CHAIN FLEXIBILITY

Flexing of polymer chains occurs as a consequence of rotation about bonds in the polymer backbone and the greater the degree of rotational freedom, the greater the degree of polymer chain flexibility.

Chain flexibility has a major effect on the glass transition temperature of polymers and the relationship between glass transition temperature and rate of polymer hydrolysis has already been discussed. However, polymer chain flexibility also has an important effect on

the enzymatic degradation of polymers. Thus, even though many native enzymes can potentially cleave certain polymer bonds, in actual fact most *in vivo* degradation of hydrophobic polymers is a purely or at least predominantly a hydrolytic process. Perhaps the best understood case are aliphatic polyesters which have been shown to degrade predominantly, if not exclusively, by a simple chemical hydrolysis even though degradation occurs in the presence of esterases which are capable of rapidly cleaving ester bonds. The absence of enzymatic activity has been attributed to a lack of chain flexibility in the glassy or semicrystalline polyesters which does not allow polymer chain conformations where the ester bond is in a favorable position to interact with the esterase active site [17]. However, when polyester chain flexibility is increased by preparing non-crystalline polymers which are above the glass transition temperature at body temperature, then enzymatic hydrolysis does take place.

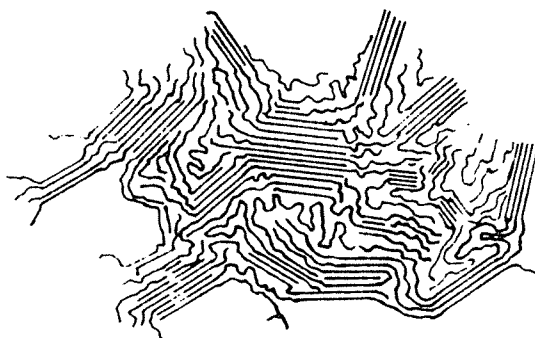
This has been demonstrated by preparing crosslinked copolymers of ϵ -caprolactone or δ -valerolactone where structural irregularity and covalent links between polymer chains prevents crystallization. When thin cylindrical devices prepared from these polymers are implanted in rabbits, rapid erosion takes place as shown in Figure 12.

Verification that hydrolysis actually occurs by enzymatic action is provided by comparing rate of enzymatic hydrolysis relative to simple hydrolysis, by noting that surface erosion takes place and by the fact that increasing crosslink density decreases rate of weight loss. Clearly because enzymes are large molecules, they can only cleave ester linkages at the surface of the device and therefore only surface erosion can take place. Further, accessibility of ester groups to the enzyme decreases as chain flexibility decreases due to increased density of covalent crosslinks. Additional evidence for enzymatic degradation is presented in Figure 13 which shows a decreased enzymatic activity when substituents are placed close to the ester links. These substituents hinder access of the enzyme to the ester bond and bulky substituents such as a tertiary butyl group almost totally inhibit enzymatic activity.

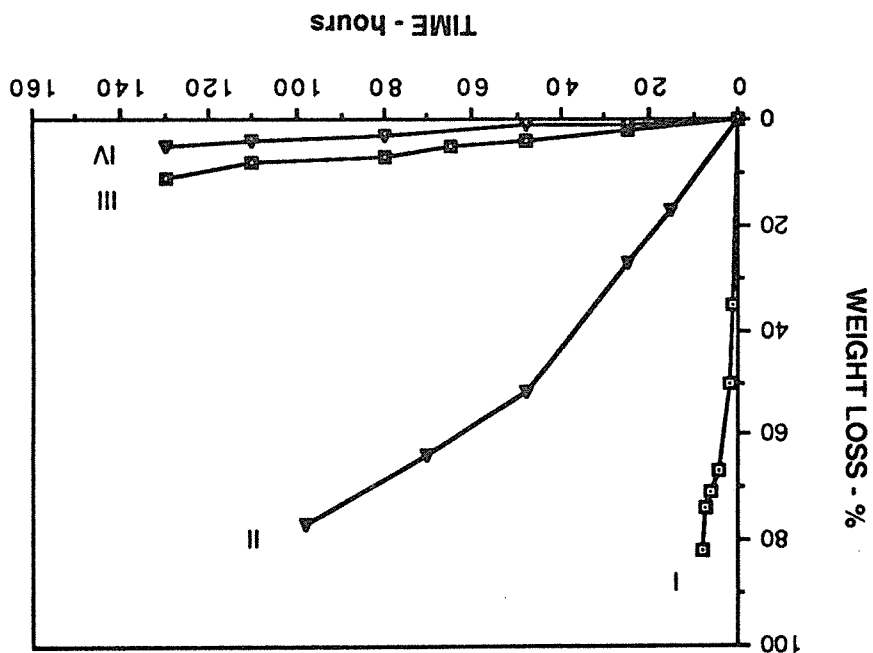
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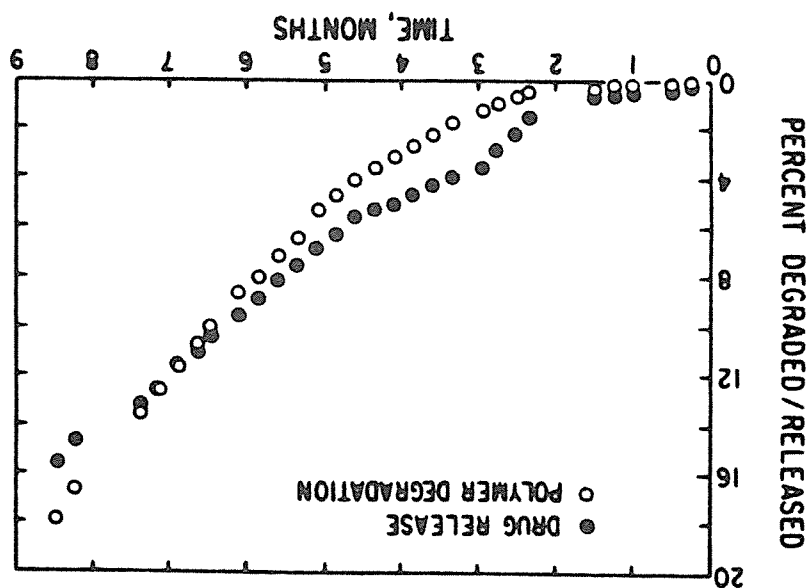


Fringed micelle model of the crystalline-amorphous structure of polymers

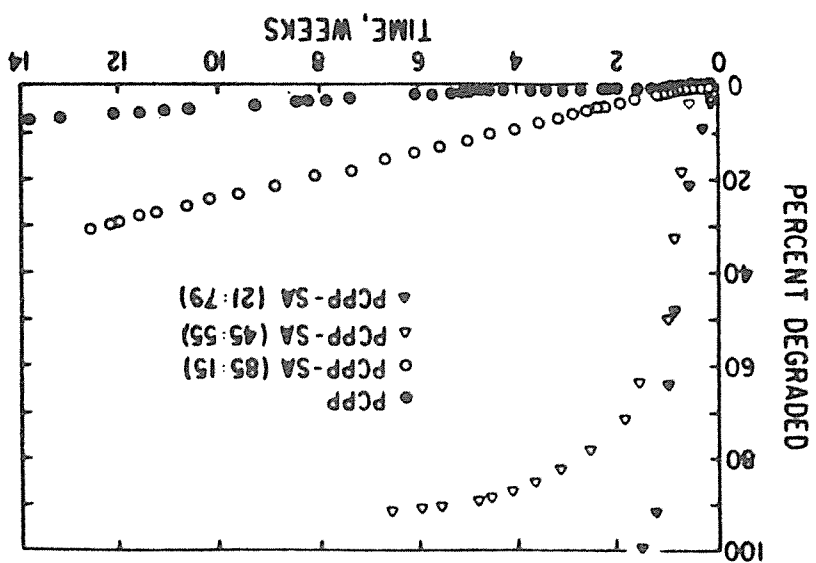


Weight loss of various poly(anhydrides) in 1N NaOH. (I) Poly (sebacic anhydride), (II) Poly[bis(p-carboxyphenoxy) methane anhydride], (III) Poly[bis(p-carboxyphenoxy) propane anhydride], (IV) Poly(ethylene terephthalate). [Adapted from Ref. 2]

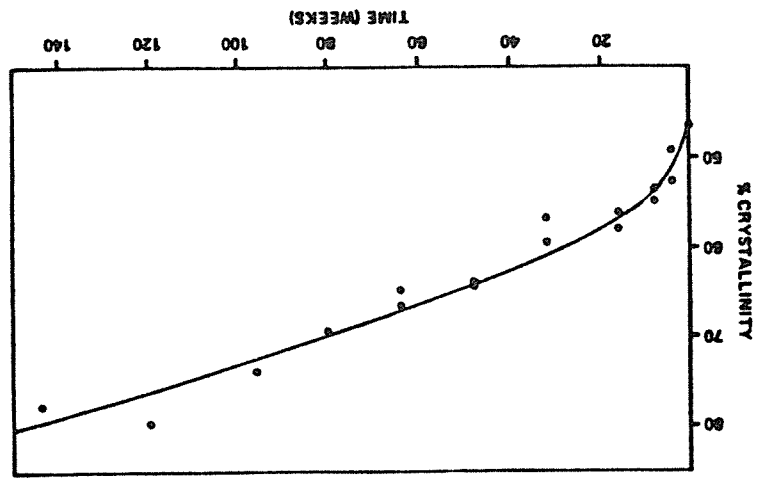
Release of p-nitroaniline from injection molded poly[bis(p-carboxyphenoxy) propane anhydride] and sebacic anhydride in 0.1 M pH 7.4 phosphate buffer at 37°C. Drug loading 10wt% [Reprinted with permission from Ref. 4]



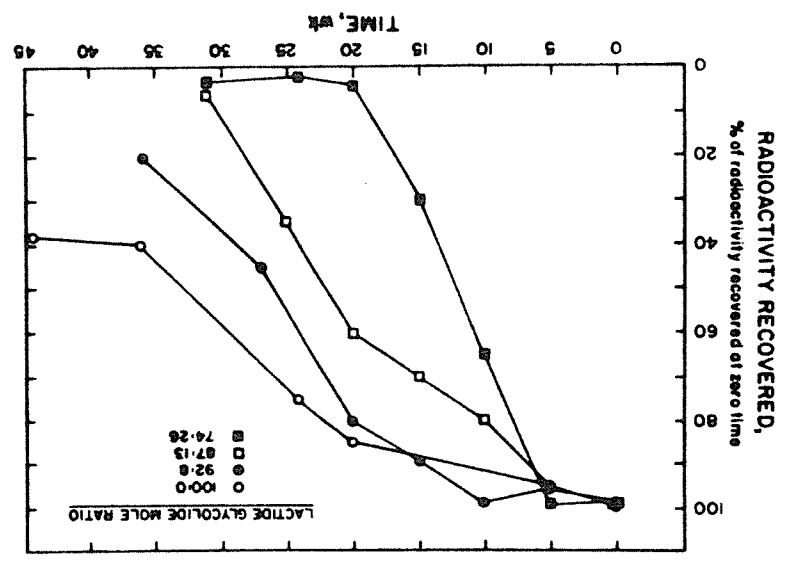
Degradation profiles of compression molded poly[bis(p-carboxyphenoxy) propane anhydride] and its copolymer with sebacic acid in 0.1 M pH 7.4 phosphate buffer at 37°C. [Reprinted with permission from Ref. 4]



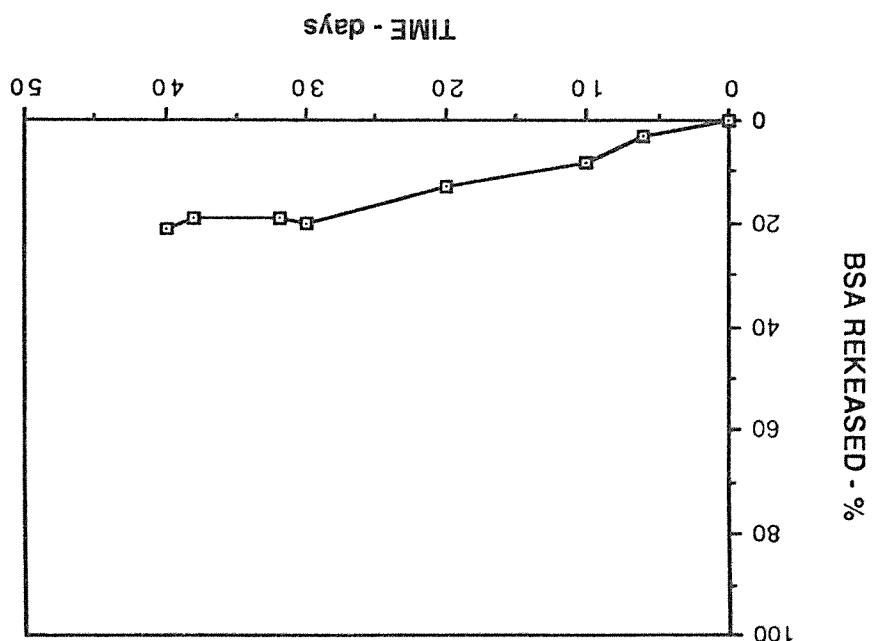
Change in the crystallinity of poly (ϵ -caprolactone) capsules, initial M_n 50,000, as a function of time in rabbit. [Reprinted with permission from Ref. 10]



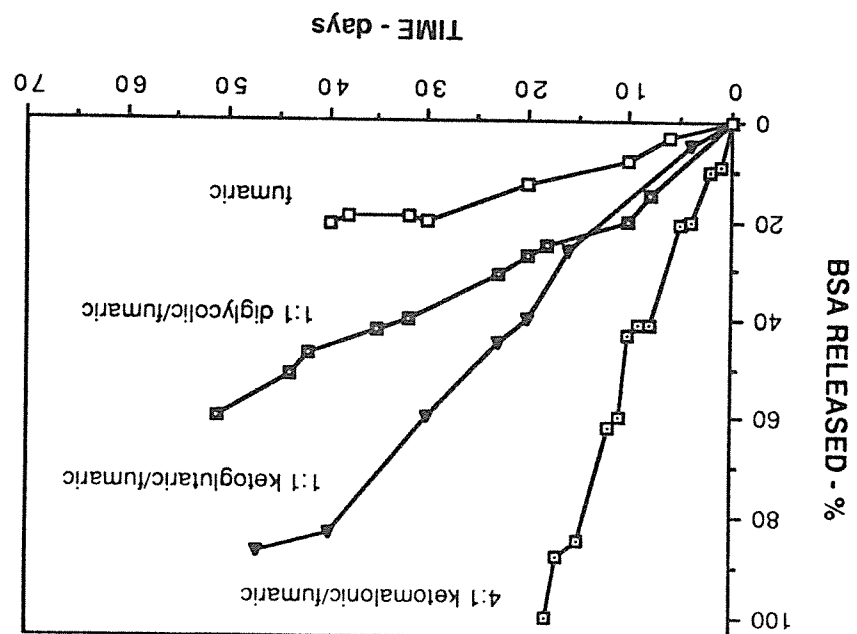
In vivo biodegradation of unstereolized norethindrone microcapsules prepared with radiolabelled poly (DL-lactic acid) and poly (DL-lactic-co-glycolic acid) copolymers. Microcapsule size 63 to 125 μ m [Reprinted with permission from Ref. 9]

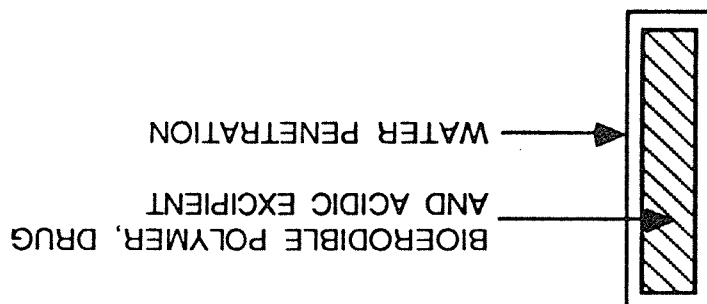


Release of bovine serum albumin (BSA) from an unsaturated polyester prepared from fumaric acid, poly (ethylene glycol) crosslinked with 60 wt% N-vinyl pyrrolidone. [Adapted from Ref. 11]

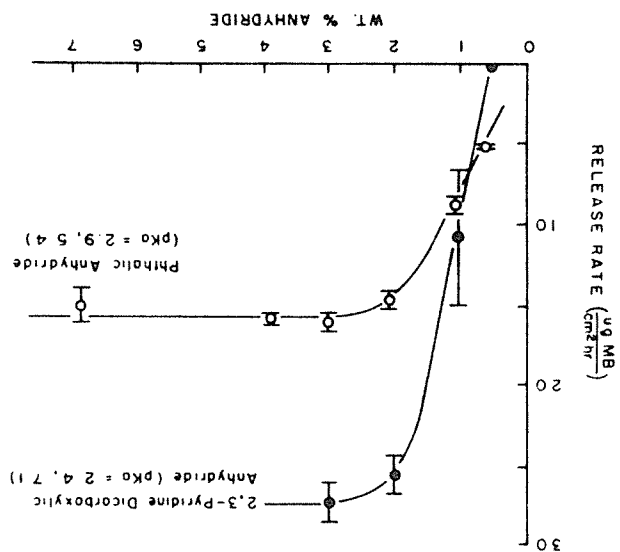


Release of bovine serum albumin (BSA) from unsaturated polyesters prepared from fumaric acid, poly (ethylene glycol) and various activated diacids, crosslinked with 60 wt% N-vinyl pyrrolidone. [Reprinted with permission from Ref. 11]

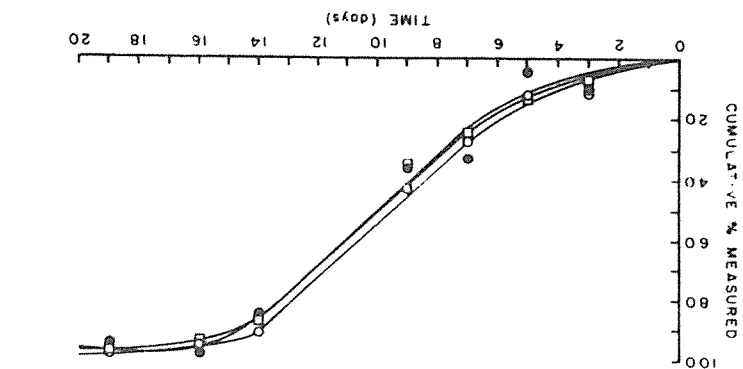




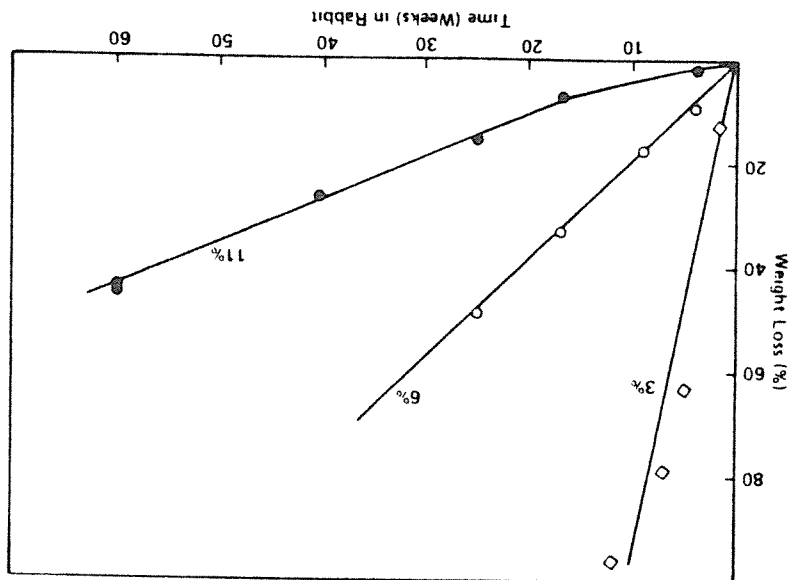
Schematic representation of surface erosion mechanism.



The effect of anhydride content on the methylene blue (MB) release rate from a poly (ortho ester) prepared from 3,9-bis(ethylidene 2,4,8,10-tetraoxaspiro [5,5] undecane) and 1,6-hexanediol / *trans* -cyclohexane dimethanol (65/35), 0.2 wt% MB, 37°C, pH 7.4. [Reprinted with permission from Ref. 14]

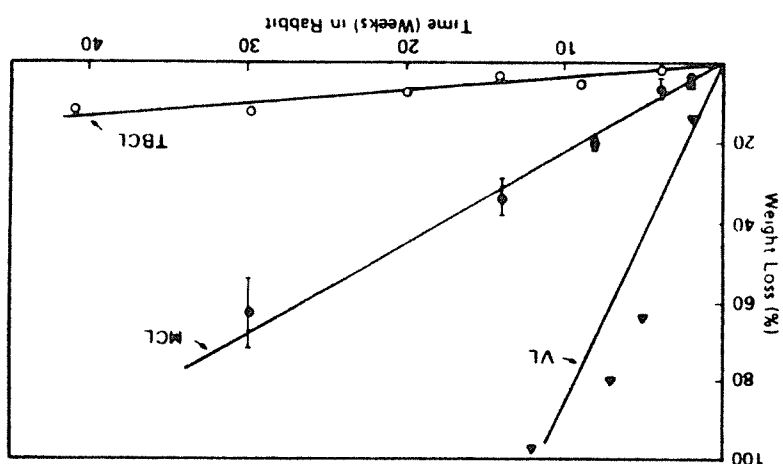


The appearance of methylene blue (MB □), ^{14}C succinic acid (●) and disk weight loss (●) in erosion of a poly (ortho ester) prepared from 3,9-bis (ethylidene 2,4,8,10-tetraoxaspiro [5,5] undecane) and 1,6-hexanediol / *trans*-cyclohexane dimethanol (50/50). Polymer contains 0.1 wt% ^{14}C -succinic anhydride and 0.3 wt% MB. 37°C, pH 7.4 [Reprinted with permission from Ref. 14]



Dependence of rates of *in vivo* surface bioerosion of copolymers of ϵ -caprolactone) and δ -valerolactone on their crosslink density ($\rho = 3, 6, \text{ and } 11$ respectively). [Reprinted with permission from Ref. 17]

Comparison of the rates of *in vivo* surface erosion of crosslinked copolymers of ϵ -caprolactone with δ -valerolactone, 6-methyl- ϵ -caprolactone, and 4-*t*-butyl- ϵ -caprolactone, crosslink density 2%. [Reprinted with permission from Ref. 17]



POLYMERS FOR VASCULAR GRAFTS

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Various cardiovascular diseases manifest themselves by blocking the lumen of blood vessels (e.g. atherosclerosis) or by weakening the vessel wall (shows as an aneurism). Progressive blockage (occlusion) of arteries may lead to a stroke, heart attack or loss of a limb through gangrene. Rupture of an aneurism in a major artery may cause almost instantaneous death. It is not always possible to simply repair the damaged sections of the vessels or to use other, less critical, vessels as autografts. As replacements for diseased vessels, artificial artery and vein grafts have immense potential for decreasing patient morbidity and mortality.

The arteries which could require replacement may vary in diameter from as little as 1 mm (digital arteries) up to 30 mm (aorta) with the majority in the 3 mm to 10 mm range. To date the only clinically acceptable artificial vascular grafts are those with a diameter in excess of 6 mm, thus limiting the application of these grafts. In order to replace satisfactorily vessels such as the coronary, femoral and popliteal arteries, artificial grafts of diameters down to 1.5 mm must be found that, ideally, remain patent for a patient's lifetime.

Current clinically used replacements for arteries include autogenous tissue (saphenous vein, internal radial artery, internal mammary artery *inter alia*), allografts (e.g. umbilical vein) and synthetic grafts (see ref 1 for a recent review). These currently successful polymer

graffs have used relatively inert polymers: expanded polytetrafluoroethylene, ePTFE (Gore® Tex®, Impra®), and polyester (Bard®, Meadox®, Vascutek®).

These materials are formed as porous tubes, the ePTFE having a microporous structure (about 30 micron internodal distance) and the Dacron® graffs being either woven or knitted. Although both materials are relatively inert in a chemical sense and are well tolerated in the body, they are mildly thrombogenic (clot forming). Dacron® is also chronically mildly inflammatory². Mechanically, the current graffs are excessively rigid in circumferential compliance.³

For these, and other, reasons these graffs only work where there is a high blood flow such as the aorta and upper femoral arteries. For vessels/graffs of less than 6mm diameter the blood flow does not appear to be sufficient to prevent thrombus formation and occlusion of the graff or production of thromboemboli (floating clots). Also endothelial cells, the normal lining of blood vessels, do not appear to grow properly on these graff surfaces.

To achieve small diameter graffs new directions are being investigated: (a) different base materials;

(b) haemocompatible or thromboresistant coatings;

(c) endothelial cell seeding;

(d) a combination of the above. Foremost, in popularity, of the materials being researched are urethane based:

poly(urethane/urea):poly(ether or dialkylsiloxane) block copolymers. These materials can be made to closely approximate the mechanical compliance of natural vessels whilst being relatively haemocompatible. The soft segment, polyether/siloxane, is usually of

molecular weight between 500 and 5000 and is rubbery at body temperature. The ratio of hard (polyurethane) to soft segment determines the bulk properties.

Other materials, designed for resorbable grafts include polyurethane/polyester copolymers and nontoxic polyesters such as polylactic acid, polyglycolic acid and polyhydroxybutyric acid.

Although the thrust of much of the material research is on single polymers that provide desired bulk and surface properties, there is considerable interest being shown in coating suitable bulk polymers with surfaces tailored more to provide certain properties such as endothelial cell adhesion/growth or anti-thrombogenicity. This is being achieved by coating the lumen surface with either biological compounds (fibrin, fibrinogen, heparin), with relatively haemocompatible polymers (such as polyhydroxyethylmethacrylate) or with specific chemical groups such as carboxyls or sulphonates.

Although many polyurethane grafts work well in animal models, none are known to have been accepted clinically yet. Major concerns are the propensity for crack formation in implanted polyurethanes undergoing cyclic stress after relatively short times. The degrees of toxicity, teratogenicity and carcinogenicity of leachable compounds, hydrolysis and oxidative degradation products are also unknown factors which mitigate against the use of polyurethanes in humans. However, the appropriate mechanical properties, together with the ease of surface modification of polyurethanes still makes this class of polymer of considerable interest.

One major area of future research is likely to be in the development of highly stable polyurethanes, thus avoiding many of the current concerns. Another major area of material

investigation that appears may be fruitful is in the development of resorbable grafts whose breakdown products are normal metabolites. These two avenues should lead to materials with suitable bulk properties that will then be surface coated to provide haemocompatibility and/or encourage endothelial cell growth.

The development of any material, composite or simple, with all desirable properties must be viewed as a long-term project. The development process must take into account the various biological constraints that have now been recognised. The material(s) used must be generally biocompatible - i.e. they must not release toxic substances and must not induce inflammation or other immune response. The blood contacting surface must be haemocompatible and must remain non-thrombogenic. The external surface of the graft must allow tissue ingrowth or adhesion to prevent the graft from moving excessively. The graft should match the mechanical properties of the natural vessel as closely as possible.

In addition, the graft may be chosen to be permanent, partially resorbable or fully resorbable. Fully resorbable grafts must not fail before they are replaced by organised host tissue which can contain blood flow, whilst permanent grafts must retain their properties for the expected life of the recipient (up to 50 years) in the hostile environment of the body. Permanent grafts also have the choice of being non-thrombogenic by means of the surface presented to the blood or by means of encouraging endothelial cell coverage of the surface.

As a prerequisite to such developmental process is the development of appropriate testing systems. These are likely to be as difficult to develop as the material itself and require the same degree of inter-disciplinary col-

laboration. Our initial step has been to examine *in vitro* and *ex vivo* methods for assessing endothelial cell adhesion to surfaces. This has involved:

- (a) establishing ovine endothelial cell lines;
- (b) developing adequate seeding techniques to cause a complete monolayer for testing;
- (c) determining appropriate shear stresses to test *in vitro*;
- (d) develop an *ex vivo* model to assay short term effects of whole blood on seeded monolayers at appropriate shear stresses. These testing systems have been used (to various stages) on PTFE, ePTFE (with and without fibrinogen adsorbed) and Nalon^{4,5} surfaces.

The harvesting and establishment of ovine carotid endothelial (OAE) cell lines is the subject of another paper. The lines are grown and maintained in McCoy's 5A medium supplemented with 20% foetal calf serum and antibiotics with the pH maintained by HEPES buffer. Cells cultured to between the 5th and 10th passage (10 to 20 doublings) are used for seeding 3mm diameter tubes of the test material. These tubes are then used in either the *in vitro* or *ex vivo* test systems described below.

Seeding is carried out by filling the tube with a suspension of OAE cells at concentration of $1 \times 10^3/\text{mm}^2$. The tube is slowly rolled (~ 10 rph) for 5h and then the tube is incubated in a flask of full medium for at least 2 days. At the end of this period the cells are labelled by incubation with $6\mu\text{Ci/ml}$ of ^{35}S -methionine for 6h.

Wall shear stresses in arteries are not exactly known although various numerical solutions and *in vivo* measurement show moderate consistency (see for example ref. 6). The time-averaged shear stress in a medium-sized artery (e.g. coronary) is likely to be in the

another 30min. At each flow rate the effluent from the tube is passed through a 1µ glass wool filter to collect any cells that may be washed off. At the end of each run the filters are dried and counted in a β-counter. The cells from both the tested half tube and the untested half tube, separately, are harvested by incubation with trypsin, the cell number counted in a haemocytometer and the radioactivity counted (after digestion) in the β-counter. This system can generate shear stresses up to 4 dynes/cm² whilst maintaining laminar flow and up to 12 dynes/cm² (est.) with possibly turbulent flow. This is of same magnitude as the calculated mean shear stresses to be found in major arteries, but does not approach the peak shear stresses that are thought to occur *in vivo*. This test system has the added advantage that a full 'mass balance' can be calculated for the cells in each run.

The *ex vivo* test system uses the sheep as a convenient test animal. An external carotid to jugular shunt (3mm id) is constructed with a connector in the centre of the external section. This shunt remains patent without anticoagulation and does not affect the animal's well-being. The section of carotid artery removed when creating the shunt is used to generate the cell line to be seeded on the test material tubes for autologous cell experiments. The tubes are tested as shown in figure 2. Flow rate is controlled by pinching the shunt downstream of the test piece and is measured by a flow probe at the commencement and termination of each flow rate. The flow probe is removed for the experiment as it causes rapid coagulation within its lumen (from blood contact with the exposed electrodes).

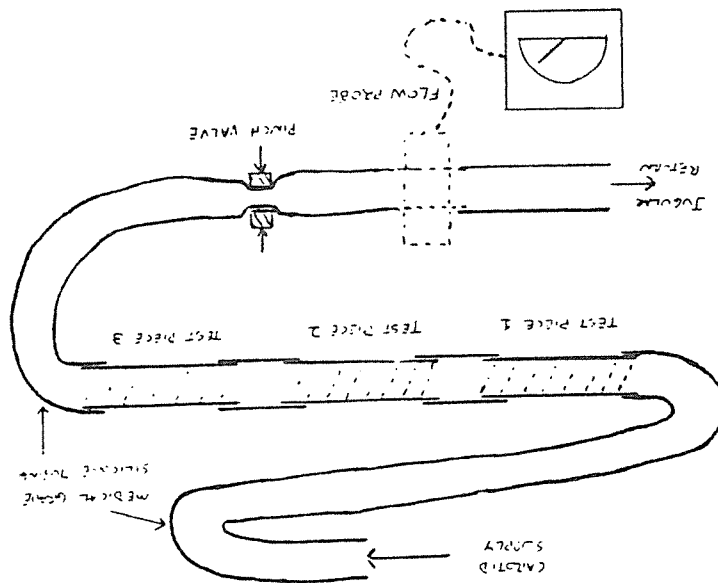
This system subjects the seeded cells to an acute test at high shear stress (ca. 56 dyne/cm²) for about 3 hours per tube. At the termination of the experiment the tested half-tube is fixed, stained for cells and

Further work is required on the testing system to improve discrimination amongst the surfaces that show strong adhesion of endothelial cells. This will include refinement of the current procedures as well as the development of more quantitative measurement of cellular parameters such as spread area, pseudopod proportion, cellular and nuclear granularity after shear stressing *in vitro* and *ex vivo*.

tested half. Early effects of whole blood without anticoagulation on the seeded cell surface and of the surface on the blood can be elucidated by this model.

A possible addition to the system is to examine the turnover of ^{51}Cr - or ^{111}In -labelled platelets at the same time.

Figure 2. *Ex vivo* test system



photographed. The fixed cells are then digested off the graft and counted in the β -counter. The number of adherent cells at the end of the test can be compared with the un-

Under development is a long-term *in vivo* model for those materials that look the most appropriate. This involves the bilateral replacement of 10 cm sections of the sheep carotid arteries with 3mm id grafts. One side is replaced with a 'control' material, usually ePTFE, whilst the other side receives an experimental material. Graft patency can be monitored by ultrasound Doppler. The sheep model is fairly unique in the fact that both carotids may be replaced by grafts as there is sufficient blood supply to the brain from the occipital and vertebral arteries in the event of failure of the carotids¹¹.

A small part of a global testing system for new vascular graft materials has been developed. Refinements and extensions of this system will be required before the appropriate assays will be available for development of the new generation of vascular grafts.

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MVE, A BIOLOGICALLY ACTIVE SYNTHETIC ANIONIC
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Maleic anhydride and vinyl ether form a cyclic
 alternating copolymer in a 2:1 ratio. Although
¹³C NMR indicated the polymer consists of a
 mixture of tetrahydrofuran and tetrahydropyran
 rings, its exact structure has yet to be
 proved. The copolymer shows a variety of bio-
 logical activities--it is an antitumor and
 antiviral agent; it has antibacterial, anti-
 fungal, anticoagulant, and anti-inflammatory
 activity; and it aids in the removal of pluton-
 ium from the liver. The copolymer is an
 immunostimulant--it induces interferon forma-
 tion; it activates macrophages, T cells, nat-
 ural killer (NK) cells, and colony stimulating
 factor (CSF); and it inhibits reverse trans-
 criptase. It has been called "the Cadillac of
 immunomodulators". A study of the effect of
 molecular weight and molecular weight distribu-
 tion on biological properties led to the syn-
 thesis of an active copolymer with low
 toxicity, which has undergone Phase I testing
 as an antitumor agent and is being considered
 for clinical evaluation in the treatment of
 AIDS.

WVE, A BIOLOGICALLY ACTIVE SYNTHETIC ANIONIC POLYMER

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The 2:1 maleic anhydride-vinyl ether cyc-
lic alternating copolymer, first reported
by Butler,¹ was initially thought to con-
sist of tetrahydropyran rings based on
proton NMR.² Kunitake, using ¹³C NMR and
calculating chemical shifts from litera-
ture values reported for related com-
pounds, concluded the copolymer consisted
solely of tetrahydrofuran rings.³ In our
work at Hercules we used the ¹³C NMR
spectra of 2,5-dimethyltetrahydrofuran-2,3-
dicarboxylic acid, 2,6-dimethyltetrahydro-
pyran-3,4-dicarboxylic acid, and 2,3-di-
methylsuccinic acid to assign the peaks in
the polymer spectrum. From this we con-
cluded that the copolymer consisted of
approximately equal parts of 5- and 6-
membered rings (Fig. 1). However, a
recent analysis has concluded that the
data available are insufficient for an
unequivocal assignment of structure.

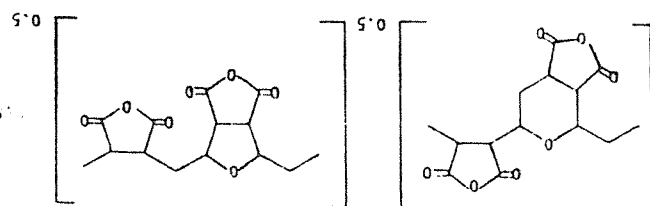


Fig. 1

The copolymer, known in the literature as
pyran copolymer or by the acronym DIVEMA,
showed a bewildering variety of biological
activities. First it was found to be an
antitumor agent; then it was found to have
antiviral, antibacterial, and antifungal
activity. It was an anticoagulant and an
anti-inflammatory agent, and even helped
to remove plutonium from the liver. A num-
ber of studies demonstrated that the co-
polymer is an immunostimulant. It is a
relatively poor inducer of interferon, and
numerous studies have shown that this has
little to do with its activity. It activ-
ates macrophages, T cells, natural killer
(NK) cells, and colony stimulating

factor (CSF). Of major importance is its ability to inhibit reverse transcriptase. It has been called "the Cadillac of immunomodulators".

DIVEMA underwent Phase I testing as an antitumor agent and was found to be too toxic for clinical use. Since it had been prepared by a procedure which led to a broad molecular weight distribution, and since it is well known that high molecular weight polymers can be toxic, a synthesis was developed to prepare copolymers with a lower polydispersity. A number were made, designated as MVE 1-5, and evaluated for activity and toxicity. MVE-2, a polymer with M_w of about 10,000 and a polydispersity about 2, appeared to have the best balance of properties, and it was selected by Adria Laboratories for further investigation. Thus, a great deal is known about its toxicity, pharmacology, and mode of administration. Unfortunately, Adria chose not to develop MVE-2, and Hercules is seeking a licensee.

At the time of this writing, MVE-2 is being considered for clinical evaluation against AIDS. Its combination of antiviral and antitumor activities should make it a promising candidate.

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ME, A BIOLOGICALLY ACTIVE SYNTHETIC ANIONIC POLYMER

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The use of synthetic polymers in medicine is growing rapidly. But in a certain sense most polymers are not used because of their biological activity. Initially they were utilized as structural materials, first as replacements for metals, but subsequently for their own favorable properties--ease of fabrication, inertness to body fluids, low cost, and ready availability. Today many different materials are in use, from heart valves to hip joints, from arteries to teeth. However, these materials are used for their biological inertness and not for their biological activity. In a similar sense, dextran and polyvinylpyrrolidone were used as plasma extenders because of their effect on osmotic pressure, and not because of any special biological activity. A considerable literature exists on the use of polymers as carriers for a variety of drugs, with the pharmacokinetics incorporated or covalently bound to the polymer.

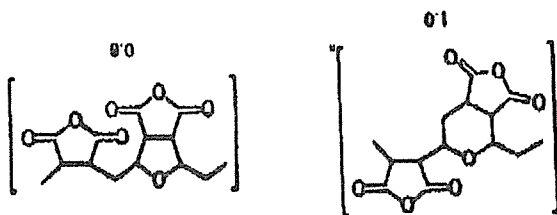
In contrast to the above mentioned applications of synthetic polymers, this discussion is concerned with biologically active polymers, particularly occurring anionic polyelectrolytes and each has its own specific biological activity. Among these are hyaluronic acid, chondroitin sulfate, heparin, agar and carrageenan. It is not surprising that interest, which centered originally on naturally occurring polyelectrolytes, has expanded recently to include synthetic polyelectrolytes as well. Some of the earlier work involved polymers of sodium ethylenesulfonate, whose polymerization was first reported in 1954. Regelson and Holland¹ found a wide spectrum of antitumor activity in mice, for the sodium salt. Unfortunately, the activity in humans appeared to be much lower and the polymer was too toxic for clinical use.² Because of our general interest in water-soluble polymers, we had prepared the 1:2 vinyl ether-maleic anhydride copolymer (DIVEMA) originally reported by Butler.³ Submission of a number of samples to the National Cancer Institute (NCI) showed that the hydrolyzed and neutralized polymers also had antitumor

Table 1. Antitumor activity of divinyl ether-maleic anhydride copolymer (NSC 46015).

Max. tol. dose/ min. eff. dose (mg/kg)	Activity	
60/10	•	Adenocarcinoma 755
32/8	•	Lewis lung carcinoma
80/10	•	Friend leukemia virus
60/7.5	•	Dunning osteoblastic leukemia
0	•	
0	•	
0	•	
0	•	

Recently, a publication appeared which indicates that more conclusive structural information is available and that the ratio of 5- to 6-membered ring structures can be controlled within certain limits by the conditions of polymerization. DIVEMA, as the hydrolyzed sodium salt, had a bewildering variety of biological activities. First, it had antitumor properties. Second, it was an interferon inducer and was active against more than 20 viruses. It also had antibacterial properties against gram-positive and gram-negative bacteria, was active against certain fungi, inhibited blood coagulation, and even aided in removing plutonium from the liver. It activates macrophages, T cells, natural killer (NK) cells, and colony stimulating factor (CSF). Of major importance is its ability to inhibit reverse transcriptase. It has been called "the Cadillac of immunomodulators". Such a wide variety of activities led, of course, to considerable work in an attempt to explain this diversity, which finally led to the not totally accepted conclusion that rather than acting directly on the organism, the polymer acts to stimulate the large white blood cells (macrophages), which scavenge and remove foreign bodies.

Figure 1. Copolymer with a ratio of 0.8:1 tetrahydrofuran to tetrahydropyran rings

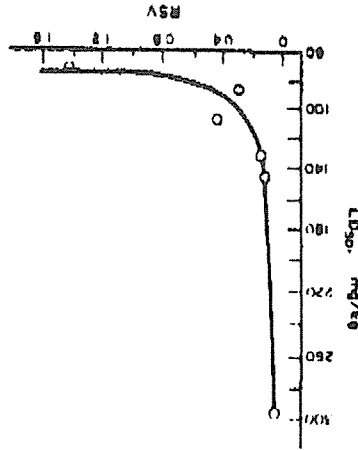


activity, but this was coupled with a much lower toxicity than had been observed with previously investigated anionic polymers. Preliminary results with one sample, designated NSC 46015, are shown in Table I. It showed activity against Lewis lung carcinoma, a slow-growing, solid tumor which is very difficult to control. The activity is comparable to that of cyclophosphamide, a widely used alkylating agent, whereas 6-mercaptopurine, an antimetabolite, is essentially devoid of activity. Because of the copolymer's rather broad spectrum of activity and its acceptable therapeutic index, NCI investigated it quite intensively. It was eventually approved for experimental clinical evaluation. DIVEMA was initially thought to consist of tetrahydropyran rings based on proton NMR. Kunitake, using ^{13}C NMR and calculating chemical shifts from literature values, concluded the copolymer consisted solely of tetrahydrofuran rings. In our work we concluded that the copolymer consisted of approximately equal parts of 5- and 6-membered rings (Fig. 1). However, after a careful analysis we concluded that the data available are insufficient for an unequivocal assignment of structure.

Based on these and other results, NCI decided to investigate DIVEMA as an antitumor agent clinically. However, it was eventually decided that the material was too toxic for use in humans. An attempt was then made to decrease the toxicity of the polymer without sacrificing its antitumor activity. It was hypothesized that DIVEMA, being a synthetic polymer of high polydispersity, contained high-molecular-weight material which was responsible for the toxicity, and the low-molecular-weight fractions were responsible for the activity. Using a ^{14}C -labeled copolymer, it was shown that although most of the material was rapidly excreted, a considerable amount remained in the animal for months.²⁰

With these ideas in mind, a sample of DIVEMA in solution was degraded. Random degradation causes the molecular weight distribution (MWD) to narrow as the molecular weight (MW) decreases. It was found that the acute toxicity dropped precipitously as the viscosity was lowered (Figure 2).⁹ The accepted means of determining MWD or polydispersity (M_w/M_n) of a polymer is by gel permeation chromatography (GPC), but reliable results on polyelectrolytes are difficult to obtain. The polymers were therefore esterified in order to accomplish more complete characterization.²¹ MWDs were determined by light scattering, and the elution volumes as determined by GPC were plotted against the product of the MW and intrinsic viscosity. The points were fitted to the universal Benoit plot, as obtained with polystyrene standards of known MW. It was then possible to determine DIVEMA MWDs and MWDs based on polystyrene standards.

Figure 2. Acute toxicity in mice of DIVEMA as a function of molecular weight. RSV (0.1% in 1 M NaOH), LD_{50} (mg/kg i.v.)



In order to prepare sufficient material for testing, a method of solution polymerization with limiting conversion was resorted to. The resulting polymers had MWD close to the 2.0 theoretical limit. It was therefore possible to prepare a polymer of relatively low toxicity that retained its antitumor activity.²² These new polymers have been designated MVE (maleic-vinyl ether).

While all of this was going on, two important events took place. Chirigos and his co-workers at NCI showed the way to use the copolymer as an adjuvant to other

treatments rather than as a straight chemotherapeutic agent.²³ He showed that if mice with leukemia were put into remission with an alkylating agent, most of the mice would still relapse and die. However, if the mice in remission were injected with DIVEMA, most of them would be cured, even though DIVEMA alone has little or no activity against leukemia. When MVE became available, Chrigos and co-workers showed that it had similar activity.²⁴ The NCI team also used the copolymer to prepare an antitumor vaccine. Whereas irradiated L1210 (a mouse leukemia) cells did not protect mice from a challenge from live L1210 cells, a mixture of the irradiated cells and the polymer protected a large proportion of the mice, and those mice that were cured were immune to a subsequent challenge with L1210 cells.²⁵

The second event, a practical one, led to submission of one of the new polymers for FDA clearance so that it could be investigated clinically. Therefore, five MVE preparations of varying molecular weights and narrow molecular weight distributions were prepared and evaluated for toxicity and activity. MVE-2, a copolymer with a weight-average molecular weight (M_w) of approximately 10,000 and a polydispersity of about 2 was selected for further evaluation because it maintained efficacy and was low in toxicity.

MVE-2 was distributed for clinical Phase I testing in several hospitals. An investigational new drug application (IND) was then submitted to FDA. Rapid intravenous administration caused acute toxicity, presumably because of depletion of calcium from the bloodstream.²⁶ Therefore, MVE-2 was injected slowly over a period of several hours. Toxicity was low, but with increasing dosage a reversible renal toxicity was observed. New animal studies showed that even this toxicity could be avoided if the myeloid were injected more rapidly, but not all of the myeloid were injected directly into the body cavity (intraperitoneally), acted as an antitumor agent, whereas when injected intravenously (iv), it sometimes caused tumors to form.²⁹

Until recently, intraperitoneal (ip) injection was considered unacceptable clinically, but this idea has changed, and data have been collected to obtain FDA approval for evaluating the material in this fashion; incidentally, the polymer is less toxic intraperitoneally than intravenously.^{30,31} Animal work shows that results vary when the tumor burden is decreased by surgery, radiation, or chemotherapy. This variable, in addition to all the others, must be considered when investigating MVE-2's activity.³²

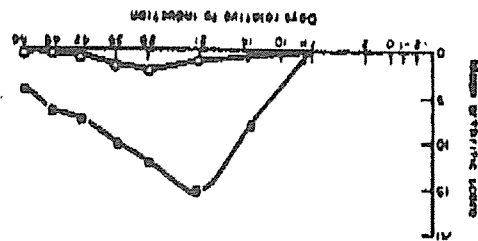
The reports of interferon induction by DIVEMA in humans led to its evaluation against a number of viruses, and it has been shown to have a broad spectrum of activity, including activity against a number of cancer-inducing viruses.³³ Figure 3 shows the protection afforded mice against two different dilutions of murine sarcoma virus (Moloney)-Plasma Variant by five daily injections.³⁸

DIVEMA has also shown antifungal activity.³⁵ Cryptococcus neoformans is a lethal yeast which attacks

It was thought that the induction of interferon was responsible for the prophylactic action of DIVEMA, since the interferon level must be built up before the infection for it to exert a protective action. However, the prolonged action of DIVEMA argues against this, since it protects long after any circulating interferon can be found in the blood stream.³⁷

It seemed to be a logical alternative that DIVEMA, and other related materials, affects the immune response of the animal, and considerable work has been done to

Figure 4. Inhibition of adjuvant disease in rats by DIVEMA. Adjuvant injection at day 0, DIVEMA at days -1, +7, +14. ■ Control (l-tanks' balanced salt solution), ○ DIVEMA.



antigens, and it is considered of importance of the similarity of the animal disease to rheumatoid arthritis.

Figure 3. Protection of mice against Moloney sarcoma virus-plasma variant by DIVEMA. Virus dilution 10^7 , □ control, ■ treated; 10^3 , △ control, ▲ treated. Reproduced by kind permission of S. Karger AG, Basel from the paper by M. A. Chirigos, Comparative Leukemia Res., 289 (1969).

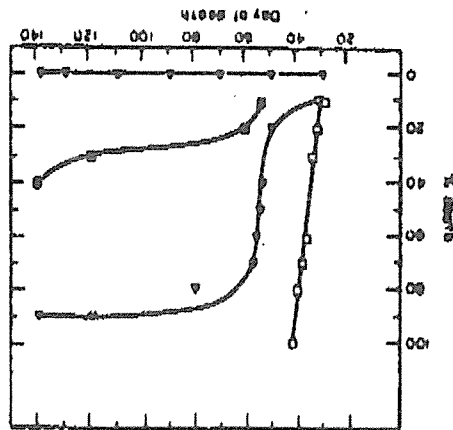


Figure 4 shows the inhibition of adjuvant disease in rats by DIVEMA.³⁶ Adjuvant disease is believed to be due to a hypersensitivity reaction to mycobacterial

19 days. er than 90 days; untreated controls had a survival time of days before challenge had a mean survival time of great-
ments of DIVEMA (25 mg/kg i.v.) either a week or several
the lungs and brain of mice. Thus, mice given two treat-

investigate this possibility. Regelson and co-workers³⁸ studied the interaction of DIVEMA with the reticuloendothelial system (RES) by investigating the effects of DIVEMA on phagocytosis in mice. Normally, foreign bodies are removed from the blood stream by a clean first-order reaction, so that one need simply determine the half-life. It is quite obvious from the results shown in Fig. 6 that the effect of DIVEMA is time-dependent. Thus, the half-life for an untreated mouse remains fairly constant at about 13.5 min for a number of foreign bodies. When colloidal carbon was injected two days after DIVEMA, the half-life doubled, i.e., the rate of phagocytosis decreased. After seven days, however, the half-life had decreased to 4 min, showing a marked acceleration compared to the control. Similar results were obtained with a lipid emulsion and with sheep red blood cells. Since the RES plays a defensive role in inflammation and immunity, its stimulation could explain the generalized activity of DIVEMA.

In contrast to this, Hirsch and co-workers³⁹ showed the effect of DIVEMA on Rouscher virus leukemia in both normal and immunosuppressed mice (Table 2). As can be seen, in both normal and immunosuppressed mice, DIVEMA caused a dramatic reduction in the virus titer. These results suggest that the action of DIVEMA was not a result of stimulating host immune response in this test.

These results, which have been confirmed in AKR mice, are of considerable importance, even if they do not shed a great deal of light on the mode of action of DIVEMA. Since immunosuppressed patients "have a markedly increased incidence of both viral infections and cancer",⁴⁰ the fact that DIVEMA is active under these circumstances may be of major importance.

Still another mode of action of DIVEMA has been reported by Papas, Fry, and Chirigos,⁴¹ who found it to be a potent inhibitor of viral RNA-dependent DNA polymerase (reverse transcriptase); examples are shown in Table 3. Bacterial DNA polymerases were activated under the same conditions. Activity appeared to increase with both increasing concentrations and increasing molecular weights of the DIVEMA samples.

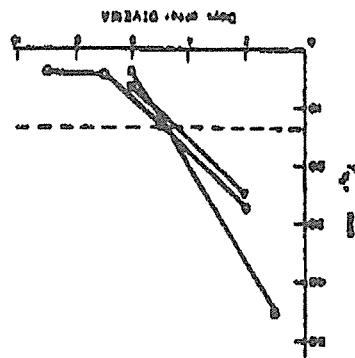


Figure 5. Rate of phagocytosis in mice as a function of time after DIVEMA administration—control (no DIVEMA); Δ lipid emulsion; $\square$ colloidal carbon; $\circ$ CFSB/C.

TABLE 3. The effect of DVEEMA on RNA-dependent DNA polymerase.

Still another instance of stimulation of host immunity was reported by Braun, et al.,⁴² who found DIVEA to be a potent stimulator of macrophage activity. Kaplan, Morahan, and Regelson⁴³ showed that peritoneal

1. (b)(1) (b)(2) (b)(3) (b)(4) (b)(5) (b)(6) (b)(7) (b)(8) (b)(9) (b)(10) (b)(11) (b)(12) (b)(13) (b)(14) (b)(15) (b)(16) (b)(17) (b)(18) (b)(19) (b)(20) (b)(21) (b)(22) (b)(23) (b)(24) (b)(25) (b)(26) (b)(27) (b)(28) (b)(29) (b)(30) (b)(31) (b)(32) (b)(33) (b)(34) (b)(35) (b)(36) (b)(37) (b)(38) (b)(39) (b)(40) (b)(41) (b)(42) (b)(43) (b)(44) (b)(45) (b)(46) (b)(47) (b)(48) (b)(49) (b)(50) (b)(51) (b)(52) (b)(53) (b)(54) (b)(55) (b)(56) (b)(57) (b)(58) (b)(59) (b)(60) (b)(61) (b)(62) (b)(63) (b)(64) (b)(65) (b)(66) (b)(67) (b)(68) (b)(69) (b)(70) (b)(71) (b)(72) (b)(73) (b)(74) (b)(75) (b)(76) (b)(77) (b)(78) (b)(79) (b)(80) (b)(81) (b)(82) (b)(83) (b)(84) (b)(85) (b)(86) (b)(87) (b)(88) (b)(89) (b)(90) (b)(91) (b)(92) (b)(93) (b)(94) (b)(95) (b)(96) (b)(97) (b)(98) (b)(99) (b)(100)

Thymectomy (day-10)	ALS (days 4-8 and -6)	DIVE MA (days -5 to -1)	Fluorocort virus (day 0)	spleen wt. (day 42)	Influenza virus (day 10)
+	+	+	+	0.151 ± 0.011	2
+	+	+	+	0.416 ± 0.070	0
+	+	+	+	0.349 ± 0.022	0
+	+	+	+	0.176 ± 0.009	2

TABLE 4. Chemotherapeutic stimulation therapy against MCAS-10

Some very exciting results are being obtained by Chirigos and Mohr at NCI.⁴⁵ In studying the dose response of DIVEA used as an adjuvant to cancer chemotherapy,⁴⁴ they have described previously (Table 4), they have

macrophages taken from DIVEA-inoculated mice were cytotoxic to B16 melanoma and Lewis lung carcinoma cells, in vitro, much more than to normal cells. The effect persisted with immunosuppressed mice, which could account for the protection afforded immunosuppressed mice by DIVEA in the work of Hirsch, et al. The use of DIVEA as an adjuvant to chemotherapy by Chirigos and co-workers¹⁴ has led to dramatic results. A Moloney lymphoid leukemia (MCAS-10) was injected into mice and allowed to grow. Then the mice were put into remission with 1,3-bis(2-chloroethyl)-nitrosourea (BCNU), a highly active alkylating agent. With no further treatment they relapsed, and only a third survived for 60 days. One treatment with DIVEA while the mice were still in remission led to 92% survival, and additional treatments were only marginally more effective (Table 4).

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It has been shown that a sample with the right viscosity and narrow MWD would not sensitize to endotoxin and would both activate macrophages and be active against Lewis lung carcinoma. Lower molecular weight samples were inactive but non-toxic, whereas higher molecular weight samples were both active and toxic.⁴⁶

Designation	(0.05 N NaCl)	0.1 mg/kg ^c	5 mg/kg ^d
X18720-91	0.17	60	70
X1871-92	0.30	60	70
X18720-71	0.70	70	70
X18720-39B	1.03	70	80
X18802-32	1.58	90	60
NSC 46015	0.76	70	100

^aTumor on day 0, BCNU on day 7; ^bAfter 81 days (controls, 0%); ^cOne injection on day 13; ^dThree injections on days 13, 15, 17.

TABLE 5. Effect of DIVEMA on L5178A mice after BCNU treatment^a

found that DIVEMA is active at extremely low levels when administered to leukemic mice put into remission with BCNU. Thus, whereas the initial study used a DIVEMA dose level of 20 mg/kg in mice, they have now found activity at levels as low as 0.1 mg. Even though toxicity would be of much less concern at the 0.1 mg/kg level in humans than at the dose of 10 mg/kg used in the original evaluation of DIVEMA, it was then found that the lower molecular weight, less toxic copolymers also have good activity (Table 5).

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